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Special Supplement to Accompany January Issue

In January the A. M. A. JOURNAL OF DISEASES OF CHILDREN will publish a special supplement containing "The Prevention of Mental Retardation: A Survey of Research," by Richard L. Masland, M.D., Research Director, National Association for Retarded Children, and Professor of Neurology, Bowman Gray School of Medicine.

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1. Breslow, Lawrence, "A Clinical Approach to Infantile Colic," J. Pediatrics **50** 196 (1957). A copy will be sent on your request.

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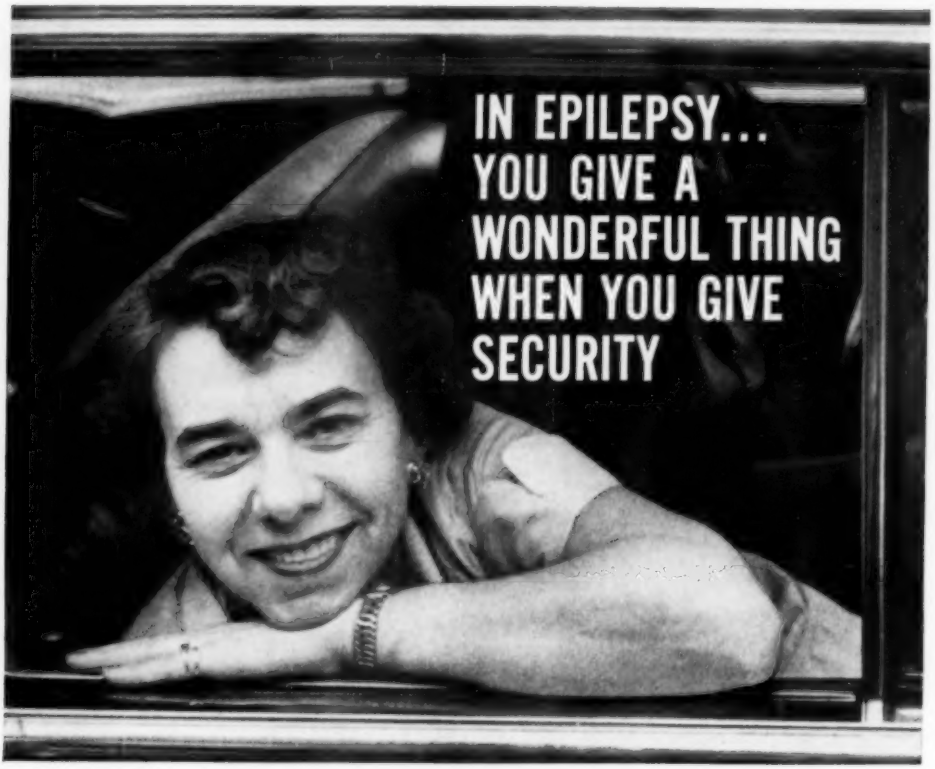

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(1) Smith, N. J., and Roselle, S.: J. Clin. Nutrition *1*:275, 1953.

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Coagulation Studies in the Newborn Period

Alterations of Thromboplastin Generation and Effects of Vitamin K in Full-Term and Premature Infants

ARTURO J. ABALLÍ, M.D.; VALERIANO LÓPEZ BANÚS, M.D.; SERGIO de LAMERENS, M.D., and SIMÓN ROZENGVAIG, M.D., Havana, Cuba

The mechanism of blood coagulation has been the subject of extensive investigations in the last two decades. As a result of these studies much valuable information has been obtained, which has modified previous concepts considerably. In spite of the advances in the field there are still gaps in our knowledge, which suggests that present theories are probably temporary. With such limitations, we may accept a rather simple hypothesis which divides the coagulation process into three stages: thromboplastin generation, prothrombin conversion to thrombin, and transformation of fibrinogen into fibrin (Table 1).

The best-known factors which participate in the second and third stages have been thoroughly studied in the blood of the normal newborn and of the premature infant. In the first stage few investigations have been performed, but they prove conclusively the existence of definite alterations. Van Creveld et al.[1] were the pioneers in this particular field, showing in 1954 that thromboplastin generation was definitely disturbed in premature infants and that the existent defect was due to a deficit of plasma thromboplastin component (PTC).[2] The authors further demonstrated that the PTC deficiency lasted a few weeks, approaching normal levels toward the end of the first month. In full-term infants the alterations encountered were not marked. Subsequently Hartmann, Howell, and Diamond[3] reported a definite defect in thromboplastin formation in full-term infants due to lack of PTC; their data confirmed the existence of a more marked alteration in premature infants. Mc Elfresh, Sharpsteen, and Akabane[4] also encountered a deficient thromboplastin generation in normal newborn infants, which in part was due to PTC deficiency. These authors further demonstrated that deficient thromboplastin formation persists for several months. Fresh et al.[5] and Barkham[6] have also found a low PTC content of cord blood.

The relationship of deficient thromboplastin generation and, more specifically, PTC deficiency of the newborn infant to other known deficiencies of coagulation factors during this period has not been ade-

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From the Department of Pediatrics of the University of Havana School of Medicine and the Maternity Service of General Calixto Garcia Hospital.

TABLE 1.—*Coagulation of Blood*

Stage I	Thromboplastin generation	Plasma precursors (AHG, PTA, & others) + platelets $\xrightarrow{Ca}$ thromboplastin
Stage II	Prothrombin conversion	Thromboplastin + Prothrombin conversion factors (proconvertin + proaccelerin) + prothrombin $\xrightarrow{Ca}$ Thrombin
Stage III	Fibrin formation	Fibrinogen + thrombin $\xrightarrow{Ca}$ fibrin

quately studied. Also, the possible effect of vitamin K on this lack of PTC has not been investigated. Recent reports suggest that PTC may be synthesized from prothrombin.[7-9] Also, coumarin derivatives bring about falls in prothrombin, proconvertin, and PTC [10-12] which are all corrected by the use of vitamin K.[10,11] Therefore, it appeared important to gather additional information on these aspects of the problem. The results of such studies are the basis of the present article.

Material and Methods

This investigation was performed at the maternity department of the University Hospital at Havana from August, 1956, to June, 1957. It includes a total of 82 infants (51 full-term and 31 premature). All full-term newborns appeared perfectly normal, and their ages varied between 3 and 144 hours. Many of them were entirely breast fed; others were receiving complementary feedings. Most of the premature infants were also doing well at the time of the investigations, and most of them survived. There were five in poor condition (two of which were extremely immature). The birth weights for the entire group varied between 850 and 2250 gm. Their ages varied from 3 hours to 39 days. All the premature infants were artificially fed, receiving a half-skimmed powder milk formula with added carbohydrate, but the smaller ones were starved for the first 48 hours. None of the mothers received vitamin K prior to or during labor.

In all these cases thromboplastin generation tests were performed. The method used was that of Biggs and Macfarlane,[13] and barium sulfate was employed in the preparation of "prothrombin free" plasma (PFP). The thromboplastin generation was determined after one, three, five, and seven minutes' incubation. After mixture and substrate were put together clot formation was observed for a maximum of 90 seconds. The results of these tests are reported in seconds in this article. Assays done by others reveal that 100% is equivalent to approximately 10 seconds.[14] All the glass material used in these tests was siliconized with Dowcorning 200 fluid, and the needles were treated with an oil-soluble quaternary ammonium compound (Arquad 2-C; Armour). The tests were always performed on venous blood obtained from the scalp or elbow with the technique already described by one of us (V. L. B.).[15] Oxalated blood was used for PFP; citrated blood, for platelets and substrate. A modification of the two-syringe method was used.

A normal adult control was always done simultaneously. (Blood was drawn by the two-syringe method.) A total of 52 thromboplastin generation tests were done in these adult controls.

Complete thromboplastin generation tests, with all reagents from the infants, were performed in 10 newborn infants before vitamin K administration and in 8 of these 48 hours later. This test was also done in 20 premature infants, 11 tests before and 14 after vitamin K (in 5 of these babies it was done before and 48 hours after the vitamin was administered).

In all the cases treated with vitamin K a water-soluble preparation, menadione (Kapilon), was used; the dosage was always 0.5 mg. intramuscularly.

In most of these babies, the tests were done with various mixtures with one separate reagent from the infant and all the others from the normal adult control. In Table 2 these various combinations are specified.

The determination of thromboplastin generation of infant serum alone with all other reagents

TABLE 2.—*Thromboplastin Generation Test*

All Reagents from Infants (Complete TGT)

I. Incubation mixture
   A. Deprothrombinized plasma (PFP)
   B. Washed platelet suspension
   C. Serum
   D. Calcium (0.02 M)
II. Substrate, normal platelet-free plasma

Separate Mixtures

Reagent from Infant (Coag. Factors Introduced)	Reagents from Adult Control	Deficit Investigated
1. Serum (PTC), (PTA)	PFP, platelets, substrate	PTC
2. Platelets (platelet factors)	PFP, serum, substrate	Platelets
3. Deprothrombinized plasma (PFP, AHG, PTA)	Serum, platelets, substrate	AHG
4. Substrate (prothrombin, labile & stable factors, & fibrinogen)	Serum, PFP, platelets	Second and third stage
5. Serum and PFP	Platelets, substrate	PTA (?) *

* In mixtures containing only serum of patients the PFP of controls adds enough PTA so that only PTC would be assayed. Also, with only PFP of patients the serum of controls supplies PTA. If we use a mixture of serum and PFP from the patients, any deficit of PTC, AHG, or PTA (and perhaps other thromboplastic substances) would be detected. If PTC and AHG are normal, it can be assumed that the deficit is due to another thromboplastic substance; also, if the alterations encountered with this mixture are much greater than with serum or PFP alone, some deficit in other thromboplastic substances can be postulated.

from normal adults (Mixture 1, Table 2) was performed 78 times in 49 full-term infants and in 32 instances in premature infants. Of this total of 110 PTC determinations, 68 were done before the use of vitamin K and the others were done after its administration. In 22 of the full-term infants the test was performed twice in the same baby, before and 48 hours after administration of vitamin K. For comparison, seven infants were studied twice, exactly two days apart, without any vitamin K. Also, in 12 premature infants results were obtained before and two days after administration of vitamin K.

In every instance a one-stage prothrombin time by Quick's method[14] was done. We shall refer to the results obtained with this test as "prothrombin complex activity." In our technique a commercial thromboplastin (Simplastin, Warner-Chilcott) was used. The percentage was obtained from charts prepared with various dilutions of adult oxalated pooled plasma for each lot.

Plasma recalcification times by Macfarlane's method[17] were also determined in practically all cases. In some infants Factor V (proccelerin) was also determined with Stefanini's method[18] in stored plasma.

Throughout this presentation we shall employ the abbreviations TGT for thromboplastin generation test and PFP for prothrombin-free plasma, besides the usual ones for the various thromboplastic substances.

Results

Controls.—As previously stated, controls were run simultaneously with each TGT or with each group of PTC determinations. The values showed remarkable uniformity at three, five, and seven minutes (10.2±1.63 seconds, S. E. 0.24; 11.05±2.0 seconds, S. E. 0.3, and 11.9±2.8 seconds, S. E. 0.32 respectively). At one minute there was a great deal of variation (30±18.1 seconds, S. E. 2.8). Hence, this determination at one minute is unreliable. This is accepted by everyone who works with the TGT. The values of means and the range of two standard deviations above the mean appear in Figure 1. The Quick prothrombin time in this control group was 89±11% (range, 67% to 111%), and recalcification time in plasma was 103.3 seconds.

Complete TGT.—As one would expect, the TGT's using all the reagents from the infant were much more abnormal than those

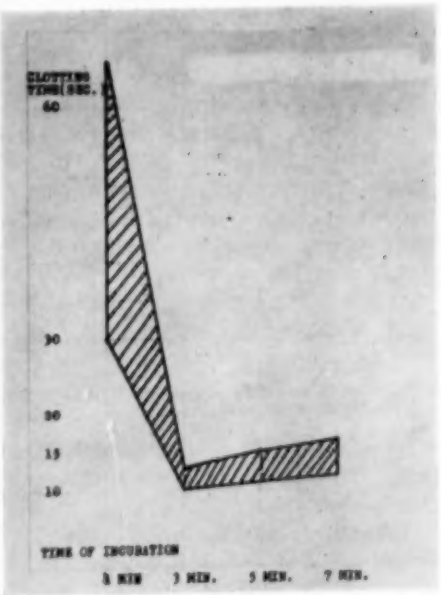


Fig. 1.—Thromboplastin generation test in controls. Mean values and two standard deviations above the mean in 52 cases.

with only single reagents (and the rest from normal adults). These results are tabulated in Tables 3 and 4. In all the cases it was at least slightly altered. This was so even though in full-term babies Nos. 2, 3, and 5 and in premature infants Nos. 12 and 18 the Quick prothrombin time was fairly normal (75% or better). The mean values for the TGT before vitamin K were 81.3, 70.3, 52.3, and 41.6 seconds for full-term infants and 71.5, 53.8, 46.5, and 47.3 seconds for premature infants. In full-term babies that had received vitamin K there was always a definite improvement. In several of them the test reached normal levels. The prothrombin complex activity also improved markedly in six out of eight. In premature infants who had received vitamin K very abnormal tests were still obtained in 11 out of 14. In the five premature infants in whom the test was performed twice, it improved in two, remained the same in one, and became worse in two. In many cases the prothrombin complex activity after vitamin K was lower in premature infants (mean,

TABLE 3.—*Complete TGT of Full-Term Infants*

Case *	Weight, Gm.	Race	Sex	Age, Hr.	TGT 1 Min.	TGT 3 Min.	TGT 5 Min.	TGT 7 Min.	Prothrombin Time (Quick), %	PRT † Sec.	Factor V, %
1	3,409	W	M	27	63	39	20	24	24	149	...
2	5,000	W	M	84	87	14.7	20	18	75	85	...
3(b)	2,950	N	M	24	78	80	35	30	75	114	75
(a)				72	90	21.5	24.5	28	48	115	75
4(b)	2,610	N	F	28	77	88	57	35	21	94	65
(a)				74	17	13	13	13	100	82	100
5(b)	3,180	N	M	33	90	51	50	59	92	88	180
(a)				81	90	71	38	28	92	62	85
6(b)	3,500	N	M	18	90	90	90	90	21	325	100
(a)				66	84	38	30	28	92	81	100
7(b)	2,720	W	F	48	90	90	58	39	35	211	75
(a)				96	65	16	20.5	18	78	90	100
8(b)	4,200	N	M	14	88	76	63	36	13	136	70
(a)				62	36	9	9.5	10.5	75	76	88
9(b)	4,100	W	M	53	90	90	90	60	59	150	...
(a)				101	27	19	19	20	107	60	...
10(b)	2,750	W	F	52	90	84	40	25	37	180	...
(a)				100	16	12	15	18	107	90	...

* (b), before vitamin K; (a), after vitamin K.
† PRT, plasma recalcification time.

67.5%) than in full-term babies (mean, 86%); however, in some cases marked increases took place.

The mean values for the complete TGT before and after vitamin K appear in Figure 2. It can be observed that the drop of clotting time after administration of the vitamin was marked in the case of full-term infants but insignificant in premature infants. In these there was only a very slight improvement in the five- and seven-minute tests.

Mixtures.—The results of various mixtures of reagents (usually single) from the infants, with the others from normal adults, have been summarized in Table 5. Abnormalities varied considerably, but serum (determining PTC) showed the greatest alteration in most instances. Thromboplastin generation activity of deprothrombinized plasma of infants (measuring AHG activity) was normal in the majority; in only four premature infants were there slight or questionable alterations of AHG. The platelets of full-term babies also showed very little change, but several premature infants had abnormal function of the thrombocytes. In 13 out

TABLE 4.—*Complete TGT of Premature Infants*

Case	Wt., Gm.	Race	Sex	Age	TGT 1 Min.	TGT 3 Min.	TGT 5 Min.	TGT 7 Min.	Prothrombin Time (Quick), %	PRT, Sec.
Before Vitamin K										
11	1,700	W	F	12 Hr.	90	37	16.5	17	41	101
10	1,395	N	M	18 Hr.	90	90	90	90	19	90
12	1,800	W	M	96 Hr.	53	18	19	20	100	150
13	2,160	W	F	108 Hr.	38	27	23	23	48	180
14	1,670	W	F	3 Hr.	30	20	20	24	63	165
15	1,530	N	F	6 Hr.	90	90	58	90	44	150
16	2,070	N	F	50 Hr.	90	90	60	28	23	150
17	1,700	N	F	36 Hr.	45	17	22	22	46	120
18	1,440	N	M	4.5 days	80	23	23	26	107	99
19	1,700	W	M	4 days	90	90	90	90	30	160
20	1,800	N	M	2 days	90	90	90	90	14	90
Means					71.5	53.8	46.5	47.3	49.4	127
After Vitamin K										
1	2,000	N	M	7 days	76	33	43	47	37	79
2	850	N	F	16 Hr.	90	90	90	90	19	70
3	1,090	W	M	36 Hr.	90	90	78	40	48	124
4	1,700	N	M	5 days	67	74	35	17	100	99
5	1,425	N	F	17 days	90	72	28	32	44	136
6	1,850	N	F	9 days	90	30	19	18	59	119
7	1,395	W	F	39 days	50	12.5	11	12.5	75	...
8	2,250	W	M	6 days	90	90	90	90	100	105
9	2,000	N	F	37 days	90	90	43	25	83	132
15	1,520	N	F	54 Hr.	90	90	90	84	59	135
17	1,700	N	F	84 Hr.	68	30	30	31	107	135
18	1,425	N	M	6.5 days	79	23	24	25	92	103
19	1,700	W	M	6 days	78	41	19	20	83	81
20	1,800	N	M	4.5 days	73	89	24	37	39	83
Means					80	56.7	44.6	41.2	67.5	108

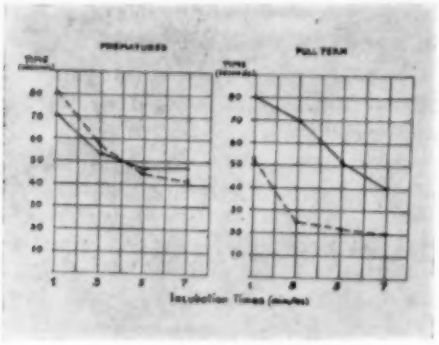


Fig. 2.—Complete TGT before and after vitamin K. Observe the difference in response between full-term and premature infants. Solid line, before administration of vitamin K; interrupted line, after vitamin K.

of 19 determinations in premature infants some alteration was found (in 6 instances, rather marked) (Fig. 3). Vitamin K had no effect on these. The nature of this particular test makes it prone to error; consequently, a larger number of observations and more detailed studies should be performed before definite conclusions are reached.

The substrate, as expected, showed alterations which were in accordance with the well-known abnormalities of prothrombin complex activity. In full-term infants it reached fairly normal values after vitamin K administration, but premature infants continued to show alterations frequently (in 50% of the cases). Abnormalities of substrate with normal prothrombin time (one-stage) were observed in two occasions, and are usually interpreted as a technical error dependent on deterioration of that substrate. The mixture of serum and PFP from infants with platelets and substrate from normal adults yielded abnormal values in 7 out of 15 cases. In these instances the degree of alteration was greater than that of the combined figures for PTC and AHG, suggesting a decreased activity of PTA (Fig. 4). In full-term babies and in most premature infants vitamin K greatly improved the results of this mixture; in two premature infants definite alterations persisted.

PTC.—With use of serum of the infants with other reagents from normal controls, abnormal thromboplastin generation was often obtained in the newborn infants (Figs. 5 and 6). Of 51 full-term babies in whom

TABLE 5.—*Thromboplastin Generation Test (Mixtures)* *

	Before Vitamin K						After Vitamin K					
	Quick Test	PTC	AHG	Plat.	Subst.	PTA	Quick Test	PTC	AHG	Plat.	Subst.	PTA
Full-term infants												
1	24	0	--	--	--	--	--	--	--	--	--	--
2	75	+	0	0	+	--	--	--	--	--	--	--
3	75	+	+	0	+	2+	48	0	+	0	+	0
4	21	4+	0	+	+	0	100	0	0	0	0	0
5	92	0	0	+	3+	+	92	0	0	+	+	+
6	21	2+	0	0	2+	2+	92	0	0	0	0	±
7	35	3+	0	0	0	3+	75	0	0	0	0	--
8	13	3+	0	0	+	+	75	0	0	0	0	0
9	39	3+	0	0	+	0	107	0	0	0	0	0
10	37	3+	0	0	0	0	107	0	0	0	0	0
Premature infants												
1	--	--	--	--	--	--	37	2+	--	--	--	--
2	--	--	--	--	--	--	19	3+	--	0	--	--
3	--	--	--	--	--	--	48	3+	0	+	--	--
4	--	--	--	--	--	--	100	3+	0	+	0	--
5	--	--	--	--	--	--	44	0	0	3+	0	--
6	--	--	--	--	--	--	59	0	0	+	+	0
10	19	2+	+	+	+	+	--	--	--	--	--	--
11	41	2+	+	+	+	0	--	--	--	--	--	--
12	100	0	--	--	--	--	--	--	--	--	--	--
13	48	2+	--	--	--	--	--	--	--	--	--	--
14	63	0	0	0	--	0	100	0	--	--	--	--
15	44	3+	0	--	2+	+	59	2+	+	+	2+	0
16	23	4+	+	±	2+	2+	--	--	--	--	--	--
17	46	±	0	0	±	0	107	0	0	2+	0	0
18	107	0	0	0	--	0	92	--	--	--	--	--
19	14	4+	0	±	2+	--	39	2+	+	3+	2+	0
20	39	2+	±	2+	2+	+	63	0	0	0	--	0
8	--	--	--	--	--	--	100	3+	0	4+	+	2+
9	--	--	--	--	--	--	53	0	+	2+	0	+

* Extent of alteration reported as 1+ to 4+.

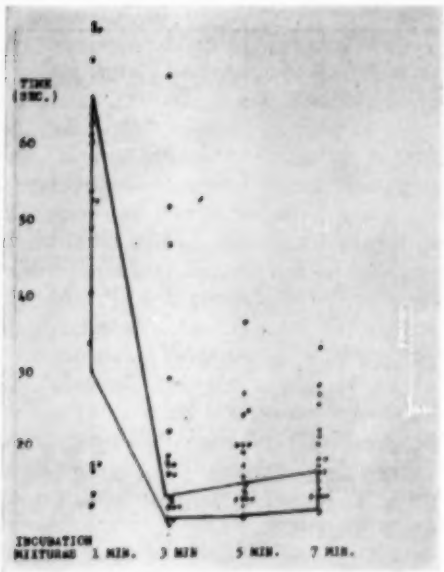


Fig. 3.—TGT with platelets of premature infants and other reagents from controls. About half of the values are abnormal. The area included within the continuous lines represents values within two standard deviations of the mean for controls.

the test was performed prior to administration of the vitamin K, 17 (32%) showed marked alterations and only 13 (25%) were within two standard deviations of the normal means for adult controls. The mean values for the group were 54.3, 30.4, 23.1, and 22.2 seconds. The average time after birth for the 51 cases was 48 hours. Results appear in Table 6 and in Figures 5 and 6.

The values of the serum TGT obtained at different times were analyzed (Table 7), and a definite increase of clotting time was observed toward the third day. Although significance cannot be proven by statistical analysis, the curves constructed with three-, five-, and seven-minute values were parallel to that of one-stage prothrombin times obtained in 141 newborn infants (Fig. 7).[19] By arranging cases by groups according to their prothrombin complex activities a very definite relationship could be established (Table 8). In the 18 cases with one-stage prothrombin of 65% or over only 7 had a somewhat delayed thromboplastin generation of the serum, whereas of the 13 infants whose prothrombin levels were between 35% and 64% thromboplastin generation was abnormal in 8 and of the 17 infants with less than 35% there were 15 who had markedly altered serum thromboplastin generation.

The effect of vitamin K agrees with the above data. Of 27 cases where the TGT with infants' sera was performed 48 hours after the administration of the vitamin, the means fell well within two standard deviations of the normal adult controls in all but five infants. In no instance was the clotting time markedly abnormal after three minutes'

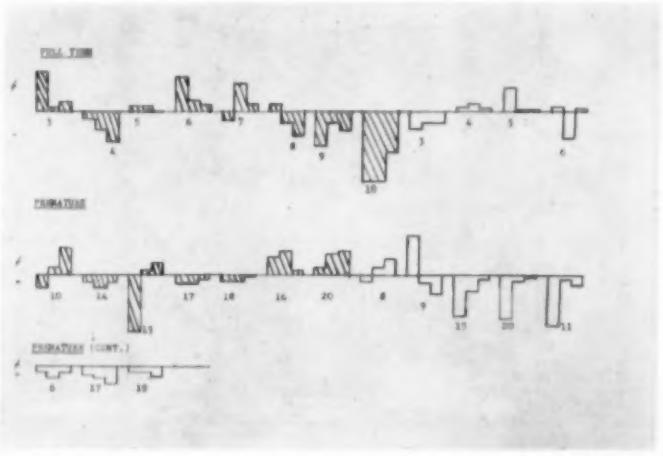


Fig. 4.—PTA in newborn infants (full-term and premature). Differences between serum-PFP mixture and sum of differences (PTC-control and PFP-control). Results above baseline suggest deficit of PTA (or other thromboplastic substances). The numbers indicate the patients' numbers in Tables 4 and 5. Hatched bars indicate values before administration of vitamin K; open bars, after vitamin K.

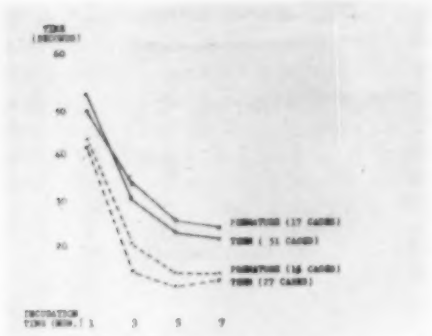


Fig. 5.—PTC mean values before and after administration of vitamin K. Continuous line represents curves before vitamin K; interrupted line, after vitamin K.

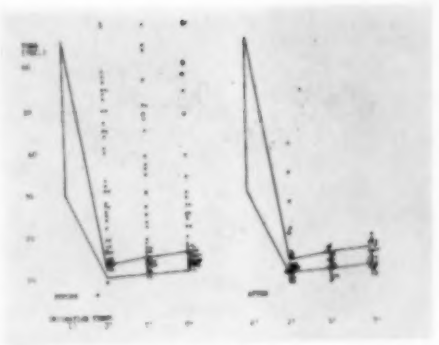


Fig. 6.—Scattergraph of PTC values in full-term infants (before and after vitamin K). Notice the normalization of most of the tests. Area included within continuous lines represents values within two standard deviations of the mean for controls.

incubation. The mean age at which the tests were performed in the treated group was 85 hours. However, even the values obtained at 99 hours without vitamin K were much more abnormal than those of infants who had been given the vitamin (Table 6). Also, of 22 infants in whom tests were performed before and 48 hours after the vitamin there was a definite improvement of the mean values in 19. Of the seven infants in whom the tests were repeated 48 hours later without vitamin K administration, the TGT of serum improved in accordance to the rise which seems to occur in the newborn after the third day, but the difference was not nearly as marked (Fig. 8). The average values failed to drop to normal levels; moreover, in three out of seven the thromboplastin generation became somewhat more altered instead of improving.

From the above data we can conclude that PTC is abnormal in many full-term infants but that marked variations may be encountered. The decrease in PTC appears to follow a curve similar to that known to occur in prothrombin complex activity, reaching the lowest values around the third day of life and rising spontaneously thereafter. Although exceptions have been encountered, alterations of thromboplastin generation of the serum (taken as PTC deficit) are very closely related to the level of prothrombin complex activity. As would be expected from such an observation, the use of vitamin K, which is known to increase the level in one-stage prothrombin activity, also brings about a marked improvement of

TABLE 6.—*PTC (Mean Values)*

	Cases, No.	PTC (Mean), Sec.			
		1 Min.*	3 Min.	5 Min.	7 Min.
Full-term without vit. K	51	54.2	30.4	23.1	22.1
Full-term with vit. K	27	43.4	14.9	12.2	13.1
Prematures without vit. K	17	50.0	34.5	25.6	25.2
Prematures with vit. K	15	43.3	21.6	14.7	14.6
2 S. D. above normal controls	52	63.0	13.5	15.0	16.5
Means at 99 hr. without vit. K	12	...	24.4	20.5	21.7
No vitamin K	7	...	20.3	21.6	21.6
No vitamin K 48 hr. later	7	...	19.0	17.7	17.8

* Time of incubation.

TABLE 7.—*PTC Without Vitamin K in Different Periods (Full-Term Infants)*

Period, Hr.	Mean, Hr.	Cases, No.	PTC, Sec.			
			1 Min.	3 Min.	5 Min.	7 Min.
0–24	13.4	14	52.0	27.6	23.2	18.5
25–48	34.3	16	57.3	33.2	23.6	22.3
49–72	60.0	9	51.0	35.6	30.8	27.2
Over 72	99.0	12	55.0	24.4	20.5	21.7

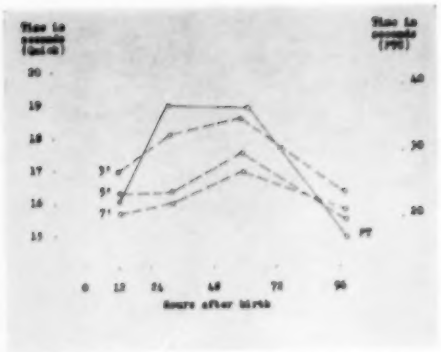


Fig. 7.—Curves with results of the TGT of serum (PTC) at three, five, and seven minutes compared with one-stage prothrombin times in the newborn period. Notice the parallelism between PTC and activity of prothrombin complex as well as the tendency to spontaneous improvement of PTC values. Solid line indicates prothrombin times (Quick); interrupted lines, the TGT of serum (PTC).

PTC deficit. In most instances normal values are obtained 48 hours after the use of 0.5 mg. of a water-soluble preparation.

In well premature infants similar observations have been encountered, but the effect of vitamin K is less evident (Fig. 9). Although differences of means appear fairly similar (Fig. 5 and Table 6), the scatter graphs show a definite difference (Figs. 6 and 9). The values at three minutes vary considerably (Table 6). Whereas in full-term babies vitamin K brought the figures down from 30.4 to 14.9 seconds, which is almost within two standard deviations from the mean for normal adults, in premature infants it dropped to only 21.6 seconds, which is definitely outside three standard deviations from the normal adult mean. This is an obviously abnormal figure. The probability that this value would be obtained by chance is less than 1%. This failure to control PTC deficiency by vitamin K in many premature infants is another strong piece of evidence that these infants often do not utilize the vitamin with maximal efficiency. In some of our cases (premature infants Nos. 4 and 8, Table 5) PTC deficiency persisted in spite of normal one-stage prothrombin values.

Comment

Although publications on the thromboplastin generation test in the neonatal period are scarce, discrepancies have been encountered. In the study of van Creveld et al.[1] most values in 18 full-term infants came within two standard deviations of normal adult values. In contrast, McElfresh et al.[4] found that practically every one of 23 full-term infants had definitely abnormal thromboplastin generation. Our results fall between these two extremes. Also, the mean values for normal adults show variations in these investigations. These differences are most probably dependent on the techniques employed. The Dutch investigators utilized capillary blood from heel prick, whereas all others have employed venous blood. Also, dilutions of serum and prothrombin-free plasma in isotonic saline were used. Both van Creveld and his colleagues[1,2] and McElfresh et al.[4] have used serum and PFP of the infants and platelets and substrate from normal adults. In our study we have also used the above mixture as a special test, but we have usually taken all reagents from infants or else have substituted single reagents and have taken all the rest from the controls (Table 2). The mean values and range of variation in our controls are in strict accordance with those generally accepted.[14]

TABLE 8.—*PTC and Prothrombin Time (Quick) in Full-Term Infants*

	Cases, No.	Alterations	Prothrombin Time, Sec.	PTC, Sec.		
				3 Min.	5 Min.	7 Min.
Without vitamin K						
High prothrombin (65% or above)	18	7	76.4	18.4	13.9	14.7
Medium prothrombin (35%—65%)	13	8	44	29.0	19.5	17.4
Low prothrombin (below 35%)	17	15	20	47.0	39.8	38.3
With vitamin K						
Moderately elevated	12	0	69	13.8	12.2	13.8
Very high	16	0	101	14.9	12.2	12.7

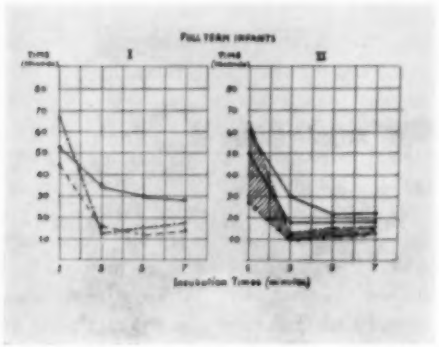
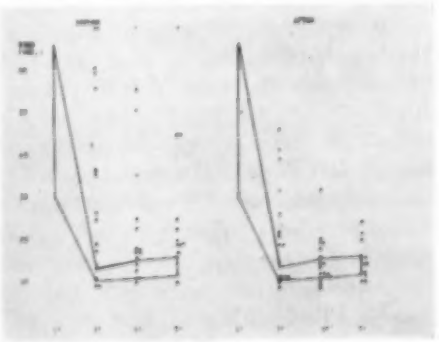


Fig. 8.—PTC before and after vitamin K in full-term babies. Notice the marked improvement, with values of the treated cases approaching very closely the range of two standard deviations above the mean. On the right are represented the results obtained in seven infants in whom the tests were repeated 48 hours apart. The second group shows an increased activity but the values remain definitely outside of the range of two standard deviations. Solid line indicates values before vitamin K; interrupted line, after vitamin K; broken line with dots, mean values of adult controls; shaded area, two standard deviations above the mean of adult controls.

In spite of the discrepancies reported it can be accepted that abnormal thromboplastin generation is often found during the neonatal period, both in full-term and in premature infants. There is universal agreement that the main deficiency corresponds to a factor present in serum, which in the case of premature infants has been definitely shown to be PTC and in full-term babies is presumably the same. However, the alterations encountered by us in this aspect are not constant, as fairly normal values were found in 25% of the babies. Barkham [6] has obtained similar results in cord blood.

Other investigators have not studied the relationship of the PTC deficit of the newborn infant to the known alterations of the second stage of coagulation—low prothrombin and proconvertin. In this study we have shown that the deficit of PTC is closely related to the prolonged Quick prothrombin time, which is known to depend on low levels of these factors. It responds similarly to vitamin K administration and even appears to follow a similar curve during the neonatal period. This is a situation very different from that encountered in Christmas disease, where the affected person is unable to maintain adequate levels of PTC in spite of normal values for the prothrombin complex. However, in coumarin poisoning a similar situation to that encountered in these newborn infants occurs, and a drop in PTC associated with a decrease in prothrombin and proconvertin takes place.[10,11] Also, a marked diminution of PTC has been found in patients with vitamin K deficit due to poor fat absorption.[11,12] In these conditions PTC activity rapidly increases toward normal following administration of vitamin K.[11] As previously mentioned, very recently evidence has been offered that PTC is formed from prothrombin. Alkjaersig, Abe, Johnson and Seegers [7] in 1955 and Seegers and Johnson [8] in 1956 showed that Factor VII and Christmas factor (PTC) were derived from purified prothrombin. Also, Spaet [9] has produced evidence in favor of the formation of PTC from true prothrombin. The present observation also favors such an interpretation. As would be expected, in the newborn infant the marked deficit of true prothrombin should lead to a decrease in PTC. Some prolonged deficit of PTC throughout the first months of life is also logical in view of the persistence of diminished true prothrombin activity.

Fig. 9.—Scattergraph of PTC in premature infants. Observe the improvement of values with vitamin K, which is much less definite than in full-term babies. Area included within continuous lines represents values within two standard deviations of the mean for controls.



Our findings offer a brighter outlook for the prophylaxis of hemorrhagic disturbances in the newborn period. Since vitamin K controls PTC deficiency secondarily to its effect on prothrombin complex activity, things remain the same as before the discovery of a definite alteration of the first stage of coagulation. However, it is possible that in some cases difficulties may be encountered in the synthesis of PTC from prothrombin, as some delay may occur. In two premature infants marked alterations of the TGT of serum were present in spite of normal values for prothrombin activity. Also, we have seen a case of hemorrhagic disease in which the one-stage prothrombin time was not markedly altered but the coagulation time and the prothrombin consumption were very abnormal.

In premature infants who had not received vitamin K we found very similar results as in full-term infants. This contrasts with the reports of van Creveld et al.[1,2] and Hartmann, Howell, and Diamond,[3] who encountered lower values. The most probable explanation is that in many of our premature infants the tests were done at a later time during the newborn period, when disturbances are less marked. It may be also due to differences in diet: All our premature infants were artificially fed, whereas many of the full-term infants were at the breast. The known prothrombin deficiency of breast-fed infants during the early neonatal period should also result in lower values for PTC.

Many premature infants have shown markedly altered thromboplastin generation and PTC deficiency in spite of vitamin K. The prothrombin levels in these cases have remained somewhat lower than in full-term babies (65.7% and 86%, respectively) in spite of the fact that they were usually performed later during the neonatal period. In a previous presentation [20] we found that abnormal premature infants had a very marked difficulty in increasing their prothrombin complex activity after vitamin K and phytonadione (vitamin K_1) oxide.

Lelong et al.[21] have shown that even small premature infants that make good progress have some difficulty in raising the true prothrombin and proconvertin levels, in spite of administration of vitamin K. This offers at least in part an explanation for the persistently abnormal TGT of premature infants. However, that other factors play a part is obvious, since PTC and "one-stage prothrombin" usually improve in those that develop satisfactorily. Although our cases showed a marked improvement of PTC with vitamin K, at three minutes the values still remained abnormal.

The test we performed for PTA deficiency may be open to criticism. Others [1,2,4] have used such a mixture of PFP and serum from infants with platelets and substrate from normal controls as the main test in their studies of disturbances of the first stage of coagulation. In such a mixture any deficiency of plasmatic factors participating in the generation of thromboplastin would be detected. If the separate TGT's with serum of infant and with PFP from infants would be normal, it would indicate normal PTC and AHG. Then any alteration obtained with the mixture of PFP and serum would be due to some other deficiency. Also, if the abnormality resulting from such combination is of a greater magnitude than the sum of alterations of PTC and AHG it probably indicates an additional deficiency of a thromboplastic factor (PTA or others). Our results suggest that there is a decrease of this factor in about one-half of newborn infants (full-term or premature). Koller [22] has found that Factor X (which is regarded as identical with PTA) [23] is decreased by coumarin-like anticoagulants and that in the newborn phytonadione oxide brings about an increase in activity of this factor. Beller [23] confirmed the low values for this factor in the newborn and in cord blood. In our cases there was often improvement or normalization after administration of vitamin K. The findings are in agreement with the above-mentioned reports. Although further studies are necessary, preferably with use of

blood from patients with hemophilia C (PTA deficiency), there is suggestive evidence of a PTA decrease in the blood of the newborn infant. Probably this is related to the PTC and prothrombin complex deficits, as in coumarin drug effect, and responds similarly to vitamin K administration.

Also, in our premature infants some deficiency in platelet function seemed to occur. Van Creveld et al. have reported normal values in 10 premature infants, with use of capillary blood.[2] Even so, most of the figures obtained were slightly above the normal average. In our series of 19 determinations, 6 were markedly abnormal. The ease with which errors can occur in this particular test makes it necessary to have a much larger number of cases before arriving at definite conclusions.

The complete TGT (with all reagents from the patient), although lacking specificity, is a very good screening test for any defect in coagulation factors. The poor response of this to vitamin K in premature infants in contrast to the good effect observed in full-term babies demonstrates that there are definite differences in the mechanisms involved. The existent alteration is much more complex in premature infants.

The evidence obtained in this investigation offers further proof that prematures have difficulty in properly utilizing vitamin K and that various coagulation factors in addition to those present in full-term babies are involved in the defects which predispose them to hemorrhagic manifestations.

Summary and Conclusions

A study of coagulation was carried out in 82 infants (51 full-term and 31 premature) during the neonatal period.

Complete thromboplastin generation tests (TGT) (including various mixtures) were performed in 20 premature and 10 full-term infants. Marked alterations were encountered in most cases. Response of the complete TGT to vitamin K was very good in full-term infants and poor in premature infants.

PTC was studied in 32 instances in premature infants and in 78 newborn infants. Among these, 17 and 51 tests, respectively, were performed without vitamin K, and 15 and 27, after administration of the vitamin. Determinations of one-stage prothrombin time and calcium recalcification were performed in all these babies.

PTC deficit was encountered frequently but not invariably. It was fairly proportional to the activity of the prothrombin complex and seemed to follow a similar curve during the neonatal period. Vitamin K brought about a marked improvement of PTC deficit; in full-term babies normal figures were usually restored, but in premature infants some alteration frequently persisted. These changes are similar to those reported in secondary PTC deficit and very different from those of Christmas disease.

Possibly there is also some deficiency of PTA or other thromboplastic substance.

The basic defect in coagulation of full-term infants lies in low prothrombin content, and the decreased PTC and proconvertin (also PTA) are all related to this. Vitamin K controls all these abnormalities.

Premature infants have also the same defects, but vitamin K is less effective, particularly in pathological cases. Other defects are also present, such as increased capillary permeability and possibly altered platelet function. This combination of defects accounts for the greater tendency to hemorrhage known to exist in these infants.

Calle 17 No. 609.

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The Significance of Blood Cultures in the Newborn Period

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Septicemia during the newborn period often occurs with deceptively benign early clinical manifestations.[1] Consequently, the clinician is forced to initiate vigorous antibiotic treatment on suspicion and thus depend upon a single pretreatment culture of the blood for a positive bacteriological diagnosis. It is important, therefore, to examine the validity of information gained from single cultures of the blood in newborn infants. As septicemia during the neonatal period is still a significant pediatric problem, it seemed important to investigate the interpretation of blood cultures at this age.

Clinical Material and Methods

Consecutive normal infants born over a three-month period at Ancker General Hospital, St. Paul, were studied. The infants had no physical abnormalities, no evidence of skin lesions or infection, and no temperature elevation. "Dry" skin care was practiced during this period of time. All blood for the cultures was drawn by one of us.

Skin over the neck was prepared as follows: 1. Twenty circular strokes were made with a cotton pledget soaked with pHisoHex (a synthetic anionic detergent containing 3% hexachlorophene). 2. pHisoHex was then removed with a dry cotton pledget. 3. Seventy per cent alcohol was next applied with cotton-tipped swabs. 4. Two per cent iodine solution was then similarly applied and removed with dry swabs. Ten milliliters of blood was withdrawn from the internal jugular vein; the needle was removed from the syringe, and the blood was transferred into a flamed tube and flask. Then 2.0 ml. was placed into a sterile tube containing 0.5 ml. of 10% sodium citrate, and the remaining 8.0 ml., into a flask containing 40 ml. enriched tryptose phosphate broth (29.5 gm. Difco tryptose-phosphate, 0.3% yeast extract, 0.15% agar, and 0.002% *p*-aminobenzoic acid [PABA] in 1000 ml. of distilled water). Two pour plates were made within two hours, each containing 1.0 ml. citrated blood and 15 ml. trypticase-soy agar medium (40 gm. trypticase-soy agar base, 0.3% yeast extract, and 0.002% PABA in 1000 ml. of distilled water). The plates were poured in a dust-free room to reduce air contamination. The plates and broth flasks were observed after 24 and 48 hours of incubation at 37 C for evidence of growth. At 48 hours and 7 days the broth flask was opened and smears were made for detecting bacterial growth. Each organism found was recorded as occurring on the surface or as a deep colony and was identified. Skin cultures were taken from the area of the neck through which the needle would penetrate immediately before preparation of the skin for taking the blood cultures and after preparation of the skin by various methods. Sterile cotton swabs moistened with sterile saline were rubbed vigorously over the area and streaked upon sheep blood agar plates. The resultant growth was evaluated after 24 and 48 hours of incubation at 37 C, and the microorganisms so isolated were identified.

Results

Blood was cultured from 126 normal infants ranging in age from 1 to 5 days. The results are summarized in Table 1; 96 of the cultures were completely sterile with sterile pour plates. There was growth in a single broth flask, a coagulase-negative Staphylococcus pyogenes var. albus, but both pour plates from the same infant were

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TABLE 1.—*Results of Blood Cultures in 126 Normal Newborn Infants*

Results	Cultures, No. (Total, 126)
Broth sterile	125 * (99.3%)
Broth and 2 plates sterile	96 (76%)
Broth and 1 plate sterile	
1 surface colony in 1 plate	16 †
1 deep colony in 1 plate	8 ‡
2 surface colonies in 1 plate	5 §

* Single positive broth grew S. pyogenes var. albus (coagulase-negative); both plates were sterile.
† S. pyogenes var. albus (coagulase-negative), 14; fungus, 1; Pneumococcus, 1.
‡ S. pyogenes var. albus (coagulase-negative), 6; fungus, 1; Viridans streptococcus, 1.
§ S. pyogenes var. albus (coagulase-negative), 3; fungus, 1; diphtheroid, 1.

sterile. The remaining 125 broth flasks were sterile, but several of the pour plates had single colonies and a few had two colonies. In each such instances only one of the pair of pour plates was involved. The colonies growing on the pour plates were usually coagulase-negative S. pyogenes var. albus, Penicillium type of fungi, or diphtheroids. In view of the type of micro-organisms cultured, their appearance chiefly on the surface, and their occurrence on a single plate, it seems probable that this growth frequently occurred as a result of air contamination at the time the plates were poured. It would be expected that incidence and types of such contaminating micro-organisms would vary somewhat from laboratory to laboratory.

The effectiveness of the method of preparing the skin for culture is shown in comparing the results of 37 cultures from the neck skin before and after preparation with pHisoHex, iodine, and alcohol. The residual iodine in this series was removed with an alcohol sodium thiosulfate solution (Richardson's solution) before the postpreparation culture was taken. A generous mixed flora grew out (Table 2), in contrast to cultures of the skin after this preparation, which showed little or no growth.

Comment

Dunham [2] first suggested that micro-organisms found in blood cultures during the neonatal period might not reflect bacteremia. She obtained 115 samples of jugular vein blood for culture from 49 normal newborn infants after iodine-alcohol preparation of the skin. S. pyogenes var. albus grew in 30% of the broth cultures and 18% of the pour plates. Waddell [3] similarly cultured blood from 106 normal newborn infants, finding that 39.6% of broth cultures grew coagulase-negative staphylococci. Further, even after alcohol-iodine preparation, the skin itself was found in this study to be an important source of contamination with these micro-organisms. The available data, therefore, indicated that growth in broth from a single blood culture obtained during the neonatal period, particularly when S. pyogenes var. albus was isolated, could not be taken at face value as indicating bacteremia in the infant.

With this background, our study was undertaken to determine the frequency of positive blood cultures in ostensibly normal newborn infants. From such data it was hoped to establish criteria which might allow separation of nonsignificant cultural results from those indicative of true bacteremia. The data obtained in this study were unexpected, therefore, in that under the conditions stipulated frequency of growth in the cultures was very low. Only 1 of 126 broth cultures showed growth, and the corresponding pour plates were sterile. The growth found in occasional pour plates is probably attributable to air contamination at the time of pouring. It seems possible that this low incidence of growth as contrasted to the previous studies cited above may be related to the technique by which the skin was pre-

TABLE 2.—*Results of Thirty-Seven Cultures of Neck Skin in Infants One to Seven Days Old Before and After Blood Culture*

Micro-Organisms Isolated	Cultures Showing Micro-Organisms Before Preparation,* No.	Cultures Showing Micro-Organisms After Preparation, No.
S. pyogenes var. albus, coagulase-negative	32	0
S. pyogenes var. aureus, coagulase-positive	16	0
S. pyogenes var. aureus, coagulase-negative	6	0
Escherichia coli	9	0
Aerobacter aerogenes	1	0
Viridans streptococcus	1	0

* Skin preparation consisted of pHisoHex, iodine, and alcoholic sodium thiosulfate, in that order.

pared prior to obtaining blood. The hexachlororophene preparation employed in these studies is highly efficient in removing oily material from the skin,[4] is bacteriocidal against staphylococci,[5] and has shown promise in drastically reducing the incidence of staphylococcal skin lesions in newborn nurseries.[6,7]

The cultures of the skin, performed in an effort to determine whether the flora of the skin in these particular infants was sparse or of unusual type, showed conclusively that large numbers of micro-organisms, predominantly staphylococci, were present before preparation. This flora was virtually eliminated by the preparation technique employed. Whether this method of preparation is significantly superior to techniques eliminating the detergent step is not established by these data but is the subject of controlled studies in progress.

With the technique described, septicemia due to several varieties of micro-organisms has been detected in newborn infants during the past two years under clinical conditions. With the use of this method of skin preparation, and employing two pour plates for quantitative information, together with a broth flask for maximal sensitivity, it should be possible to make a satisfactory diagnosis of bacteremia in newborn infants on the basis of a single culture.

These studies also indicate that true bacteremia probably does not exist in a significant number of newborn infants. With the technique and procedure described the result of a single blood culture from a newborn infant may be considered significantly positive if the same micro-organism grows out in both broth culture and pour plate.

Summary and Conclusions

Blood cultures on 126 normal newborn infants were performed in order to assess the significance of a single positive culture in the bacteriological diagnosis of septicemia of the newborn. The results indicate that significant bacteremia is not frequently encountered in normal newborn infants. Preparation of the skin, prior to obtaining a blood specimen, including the use of hexachlorophene has been found to reduce skin contamination of cultures to a minimum. With use of the technique described, a blood culture from a newborn infant may be considered significant if the same micro-organism is found in pure culture in both broth flask and pour plate.

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Method for Determination of Serum Phenylalanine with Use of the Kapeller-Adler Reaction

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The discovery that serum phenylalanine is increased in the metabolic disease phenylpyruvic oligophrenia has made desirable the availability of a relatively simple and accurate method for its determination in serum. This has become even more important since the observations that urine phenylpyruvic acid, which has been the commonly used test for this disease, may not be detected until the serum levels of phenylalanine exceed about 15 mg. per 100 cc.[1] and that infants less than one month old with this disease may not show the presence of phenylpyruvic acid in the urine, whereas the serum phenylalanine is elevated within five days.[2]

Microbiological methods [3] have been used, but these do not meet the requirement of simplicity. Udenfriend and Cooper [4] have proposed a chemical method in which the phenylalanine is enzymatically decarboxylated to phenylethylamine by use of an extract of Streptococcus faecalis and then the phenylethylamine is quantitated photometrically by reaction with helianthin (methyl orange). This method requires preparation of the bacterial extract. Most of the chemical methods for determination of phenylalanine have been modifications of the Kapeller-Adler test [5,6] in which the phenylalanine is nitrated and the nitrated product is determined photometrically with hydroxylamine. Armstrong and Tyler [7] modified the Kapeller-Adler test for serum, making a trichloroacetic acid filtrate, removing the trichloroacetic acid by ether extraction, and then developing the color as recommended by Block and Bolling.[6]

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We experienced two difficulties with the technique of Armstrong and Tyler: 1. It was practically impossible to remove the trichloroacetic acid by ether extraction because of the unfavorable partition coefficient. 2. Sugars and other organic constituents of serum are not removed and become charred in the analysis. In the case of sera with elevated phenylalanine levels this resulted in final colors which visually did not match the color of standards. It was found that precipitation of proteins by picric acid, followed by isolation of the amino acids by the ion-exchange resin Dowex 50-X8 and then application of the Kapeller-Adler color reaction to the eluate, provides a technique devoid of the two objections raised. Furthermore, the method to be presented requires 1 or 2 cc. of serum, whereas that of Armstrong and Tyler uses 10 cc., a quantity undesirable in pediatric practice.

Method

Reagents.—The following reagents are used: 1% picric acid; 20% KNO_3 in concentrated H_2SO_4 (dissolve salt by gentle heat); 30% hydroxylamine hydrochloride; concentrated NH_4OH, used cold from the refrigerator; phenylalanine standard, 36 mg. per 100 cc. of 0.1 N HCl (1 cc.=0.36 mg.); Dowex 50-X8, 200-400 mesh, analytical reagent grade.

Procedure.—1. Place 10 cc. 1% picric acid in a 50 cc. Erlenmeyer flask. Add slowly, with constant mixing, 2.0 cc. of serum or plasma. If the supply of serum is limited, the test can be done with 1 cc., adding 1 cc. of water to make up the volume difference and multiplying the final result by 2. Let stand five minutes; transfer to a centrifuge tube, and centrifuge.

2. Prepare column as follows: Suspend resin in water and pour into a small column (10 mm. internal diameter), in the bottom of which has been placed a glass wool plug. The column is

packed by gravity to a depth of about 5 cm., and then a glass wool plug is placed on top of the resin.

3. Acidify 9 cc. of the protein-free supernate to pH 1 to 2 (pH paper) with N HCl, and then pass it through the resin column (the level of the liquid is never allowed to pass the upper surface of the upper glass wool plug at this or any subsequent step). Wash the column with 10 cc. 0.01 N HCl and then with eight 5 cc. portions of distilled water or until the eluate is colorless (free of picric acid). This step can be hastened by applying suction to the column (insert the column in a one-hole stopper and place in a suction flask).

4. Elute the amino acids with 15 cc. 2 M NH_4OH, discarding the first 3 cc. of eluate and collecting 12 cc. in a graduated tube.

5. Transfer the 12 cc. of eluate to a porcelain dish and evaporate to about 2 cc. on a steam bath. Transfer to a test tube calibrated at 5 cc. and evaporate to dryness in a boiling water bath under nitrogen or dry compressed air.

6. Set up an empty tube calibrated at 5 cc. and labeled "reagent blank." To another calibrated tube, labeled "standard," add 1.0 cc. phenylalanine standard (0.36 mg.). Dry the standard as in Step 5.

7. To each tube add 0.4 cc. of the KNO_2-H_2SO_4 reagent and place in boiling water for 20 minutes.

8. Cool tubes in ice water, add 2.5 cc. distilled water, and allow to cool in the ice water.

9. Add 0.5 cc. of the hydroxylamine reagent, mix and let sit in the ice water for 5 minutes. Dilute with cold ammonia to 5 cc., and mix. Let stand at room temperature for 15 minutes and read the absorbances of the unknowns and standard against the reagent blank at 555 mμ. Check the cuvets for bubbles, since these may form, especially if the solution is exposed to the heat of the light beam for any extended period of time.

Calculation:

$\frac{A_u}{A_s} \times 24 =$ mg. phenylalanine per 100 cc. of serum.

Investigation of Method

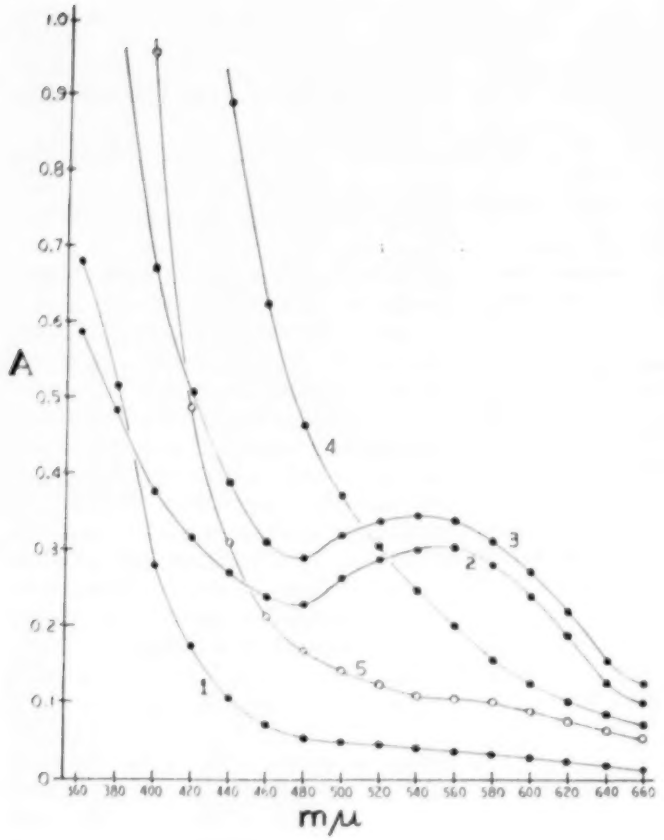
Standards and Reagent Blanks.—The absorbance of color reagent blanks was about 0.004 against water at 555 mμ. A blank in which water was substituted for serum and run through the entire procedure gave a somewhat higher absorbance, about 0.018. For maximal accuracy, therefore, the picric acid reagent and water substituted for serum should be run through the entire procedure, although for routine analyses this would not seem necessary. The picric acid-water mixture should be run through the column for each batch of resin, however, to check on this point. Similarly, when the procedure is first set up, internal standards should be run through the entire procedure. Recoveries of phenylalanine (13 experiments) ranged from 89% to 101%, with an average of 97.5%.

Removal of Picric Acid.—Stein and Moore [8] used Dowex 2 to remove the picric acid and then adsorbed the amino acids onto Dowex 50. We found this unnecessary, since the picric acid can be effectively washed through the Dowex 50 column.

Beer's Law and Color Stability.—The color was found to obey Beer's law at 555 mμ on a Beckman Model DU spectrophotometer up to an absorbance of 1.2 (1 mg. phenylalanine, equivalent to a serum concentration of 67 mg. per 100 cc.). Beer's law was also obeyed when a Klett photometer was used with a No. 54 filter. The color intensity in standards and unknowns reached a maximum within 15 minutes, remained constant for the next 45 minutes, and then began to drop slightly.

Absorption Curves.—The Figure shows the absorption curves obtained with a normal serum (Curve 1), a 0.29 mg. phenylalanine standard (Curve 2), the serum plus 0.29 mg. phenylalanine as internal standard (Curve 3), and a 0.29 mg. tryptophan standard (Curve 4). Curve 5 was obtained on the same serum by the technique of Armstrong and Tyler.[7] All absorbance readings were made against blanks run through the entire procedure. Serum samples from cases of phenylpyruvic oligophrenia gave curves similar to Curves 2 and 3. Tyrosine gave no color in the reaction.

Stability of Serum Samples.—Stability studies were carried out on normal sera to which phenylalanine was added to a final concentration of about 20 mg. per 100 cc. Five samples showed no change in three days at room temperature, but similar samples held at room temperature for seven days showed a significant drop. Three samples to which sodium fluoride was added to give a concentration of 3 mg. per cc. showed no change in seven days at room temperature.



Absorption curves. Curve 1, normal serum; Curve 2, 0.29 mg. phenylalanine; Curve 3, normal serum plus 0.29 mg. phenylalanine internal standard; Curve 4, 0.29 mg. tryptophan; Curve 5, normal serum by method of Armstrong et al. Beckman Model DU spectrophotometer.

Comment

Values found for 11 normal adult sera by this technique (employing a column reagent blank) ranged from 1.4 to 3.3 mg. per 100 cc. The absorption curve of a phenylalanine standard shows a maximum at 550-560 mμ and a minimum at 470-480 mμ (Fig.). The curve obtained with a normal serum (Curve 1, Fig.) shows no such maximum and minimum, raising the question of the accuracy of values obtained with normal sera. Furthermore, the final color obtained with normal sera was yellow, rather than the purple of pure phenylalanine standards. The color produced with tryptophan was yellow, and, on a weight basis, tryptophan gave 69% of the absorbance given by phenylalanine. Since tryptophan was found to be completely recovered in the procedure, it would appear that the technique measures the sum of the phenylalanine and 69% of the tryptophan. The presence of tryptophan may account, at least in part, for the yellow color obtained with normal sera.

Armstrong and Tyler [7] read absorbances at 540 mμ, at which wavelength the absorbance of tryptophan approaches that of an equal weight of phenylalanine. This, plus the inclusion of color due to the charred organic material, should result in higher values. Armstrong and Tyler [7] reported normal values ranging from 3 to 5 mg. per 100 cc. and believed that this was compatible with the normal values obtained for phenylalanine and tryptophan by microbiological techniques. There is considerable variation, however, in the reported normal ranges as

obtained by microbiological methods[9-13]. The lower limit for phenylalanine has been variously reported as 0.7 to 1.5 mg. per 100 cc. and the upper limit as 1.3 to 4 mg. per 100 cc.; the lower limit for tryptophan has been variously reported as 0.7 to 1 and the upper limit as 1.3 to 3 mg. per 100 cc. Stein and Moore,[8] utilizing their ion-exchange chromatographic technique, found the normal range of serum phenylalanine to be 0.69 to 0.95 mg. per 100 cc. Hsia and Driscoll,[14] employing the enzymatic technique of Udenfriend and Cooper,[4] observed a top normal limit for serum phenylalanine to be about 3 mg. Although there is a unanimity of findings as to the general order of magnitude of the normal levels of these two amino acids, there is sufficient disagreement in the exact delineation of the ranges as to make it impossible to give an opinion regarding the accuracy of any method derived by comparison of normal ranges obtained.

The use of a filter photometer with a broad band filter having a nominal wavelength of 540 mμ results in values slightly higher than those obtained with a spectrophotometer at 555 mμ. This is presumably due to rapidly increasing absorbance in this wavelength region of the color produced by the tryptophan present.

It is probable that the accuracy of the technique presented is low in the case of normal sera, and undoubtedly the precision is poor because the absorbances obtained with normal levels are in a region of very high relative photometric error. Fortunately, however, these facts do not negate the value of the analysis for the diagnosis of phenylpyruvic oligophrenia, in which the serum levels are about 15 mg. per 100 cc. or higher,[1,3,15] values well above the sensitivity of the method. Inclusion of the serum tryptophan in the result would not appear to cause any confusion, since there is no situation known in which the serum level of this amino acid would even approach this magnitude.

Summary

A method for the determination of serum phenylalanine is presented in which proteins are precipitated by picric acid, the phenylalanine is isolated by adsorption onto ion-exchange resin (Dowex 50), and the amino acid in the eluate is quantitated by the Kapeller-Adler reaction. A study of the method indicates its validity for the chemical determination of serum phenylalanine for the diagnosis of phenylpyruvic oligophrenia.

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Hereditary Spherocytosis in the Negro

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Congenital spherocytosis is a disease characterized by a varying degree of anemia, microspherocytes in the peripheral blood smear, splenomegaly, and increased hemolysis in hypotonic saline solutions. Although probably recognized [1] and treated [2] in the latter part of the 19th century, it was first accurately described as a clinical entity by Minkowski [3] in 1900, and in 1907 Chauffard [4] first described the increased fragility of the erythrocytes in hypotonic saline. Since that time the disease has been thoroughly studied and reviewed by many workers.[5-7]

The purpose of this paper is to report a genetic study in a Negro family in which there are members with evidence of congenital spherocytosis. The literature pertinent to the Negro race is reviewed, and some conclusions are drawn as to hereditary factors involved.

Report of Cases

CASE 1.—A 10-year-old Negro boy (Fig. 1) was admitted to the Harriet Lane Home June 2, 1956, complaining of stomach ache, eye pain, and feeling tired for about one week. He was well until 10 days prior to admission, when he suddenly developed midepigastric pain, anorexia, some nausea but no vomiting, and fever (99.8 F). These symptoms persisted until admission. Because his mother noticed the onset of some pallor, she brought him to the dispensary. There was no history of any jaundice, dark urine, acholic stools, or joint pains. He denied the ingestion of or exposure to any potentially hemolytic agents. The family history revealed that a maternal uncle had had "low blood and something removed from his liver" at age 48 years (Case 5). The twin of a younger sibling

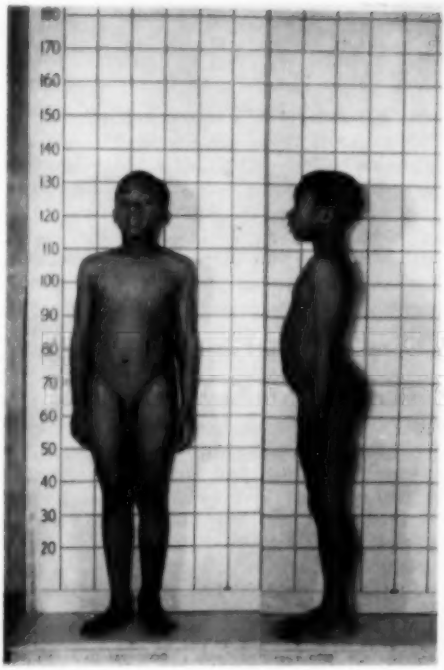

Fig. 1 (Case 1).—Body habitus.

"turned yellow and died" at the age of 5 days. Another younger sibling died at the age of 6 months at home of "pneumonia and turned stiff."

On examination he was a well-developed and well-nourished 10-year-old boy with typical Negroid features, appearing pale but in no distress. His temperature was 99.5 F; pulse rate, 120 per minute, and blood pressure, 125 mm. of Hg systolic, 75 mm. of Hg diastolic. The nail beds, palms, and soles were very pale. The sclerae were white. Ausculation of the heart revealed a Grade 2 blowing systolic precordial murmur. The spleen was palpable 1.5 cm. below the left costal margin in the midclavicular line, but the liver and kidneys were not palpable. The remainder of the examination was not remarkable.

Initial laboratory studies (Table 1) revealed a hemoglobin of 6.4 gm. per 100 cc. (Sahli), with a hematocrit value of 17%. There were 2,320,000 red blood cells and 19,000 white blood cells per

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TABLE 1.—*Laboratory Studies in Case 1*

	RBC, Millions/ Cu. Mm.	HB., Gm./100 Cc.	Hematocrit Value, %	Reticulocytes, %	Nucleated RBC/100 WBC	Spherocytes	Platelets/ Cu. Mm.	Osmotic Fragility	
								Beginning	Complete
6/1/56	2.32	6.4	17	0.6	7	++	210,000	0.60	0.50
6/4/56		7.4		15.7	12				
6/11/56		9.0	26	10.5					
6/14/56	Transfusion 300 cc. packed RBC								
6/15/56	Operation								
6/15/56	(3 hr. after operation)						1,500,000		
6/16/56			38				818,000		
6/21/56	2.62		41				523,000		
6/29/56		12.7		0.1	0	++	542,000		
7/11/56		12.8		0.4			218,000		
1/16/57	3.84	13.8	42	1.1	0		294,000		

cubic millimeter, with a normal differential count. The peripheral blood smear showed 7 nucleated red blood cells per 100 white blood cells and numerous microspherocytes. The reticulocyte count was 0.6%, and the platelet count was 210,000 per cubic millimeter. The total bilirubin was 1.6 mg. per 100 cc., with direct-reacting bilirubin less than 0.8 mg. per 100 cc. Osmotic fragility revealed beginning hemolysis in 0.60% saline and complete hemolysis in 0.50% saline. Paper electrophoresis of the lysed washed red blood cells showed the presence only of the normal adult type of hemoglobin. The direct Coombs antiglobulin test, stool examination for occult blood, and serological test for syphilis were all negative. Urine analysis, old tuberculin skin test, and unabsorbed heterophile agglutination test were normal. Roentgenograms of skull, chest, and long bones revealed no abnormalities. Abdominal x-ray showed no gallstones.

During the early hospital period he recovered from this apparent aregenerative phase of the bone marrow with a moderate reticulocytosis and increase in hematocrit value. On June 14, 1956, he was transfused with 300 cc. packed red blood cells, and the following day a splenectomy, along with removal of a tiny accessory spleen, was performed. The spleen weighed 145 gm. His platelet count rose to 1,500,000 per cubic millimeter during the immediate postoperative period, but he remained asymptomatic and recovered uneventfully. He was discharged June 24, 1956, with a hematocrit value of 41%, red blood cell count of 3,480,000 per cubic millimeter, and platelet count of 523,000 per cubic millimeter. Since his discharge he has been more energetic and has gained 5 lb., and his hemoglobin has remained 13 gm. He was last seen in January, 1957, and there was no recurrence of symptoms or signs.

CASE 2.—The mother of Case 1, a 32-year-old Negro housewife, has never been seriously ill but has noted mild yellowish scleral tint and has been frequently listless and easily fatigued.

Physical examination revealed a moderately pigmented slightly obese 32-year-old Negro woman with mildly icteric sclerae. There was a Grade 1 apical soft systolic murmur. The spleen was palpable 4 cm. below the costal margin in the midclavicular line. The liver was not palpable.

Laboratory studies revealed a hematocrit value of 27%, an osmotic fragility test showing complete hemolysis in 0.70% saline, and numerous microspherocytes on peripheral smear. Cholecystogram was normal. Paper electrophoresis of the lysed washed red blood cells revealed the presence of only the normal adult type. She is at present awaiting splenectomy.

CASE 3.*—A Negro woman, the sister of Case 2, was examined as part of the family study in October, 1956. She had no past history suggestive of hemolytic episodes. Physical examination revealed a palpable enlarged spleen. Her hemoglobin was 11.6 gm. per 100 cc., and the osmotic fragility was increased. The peripheral blood smear showed many microspherocytes.

CASE 4.—A 12-year-old Negro boy, the son of the third patient and the cousin of the first patient, was admitted on Oct. 18, 1956, because of a seven-day history of nausea, vomiting, increasing pallor, and abdominal pain. The family history revealed that an uncle (Case 6) had had a splenectomy in a Baltimore hospital.

The physical examination was unremarkable except for mucosal pallor, hepatosplenomegaly, and a mild fever (99.8 F).

The admission hemoglobin was 5.7 gm. per 100 cc.; the red blood cell count was 1,530,000 per cubic millimeter, and the white blood cell count was 6700 per cubic millimeter. The hematocrit value was 16%, and the platelet count was 312,000 per cubic millimeter. There were 23% reticulocytes. The peripheral smear revealed obvious microspherocytes. The total serum bilirubin was 1.7 mg. per 100 cc., with direct-reacting bilirubin 0.4 mg. per 100 cc. There was no sickling. Erythrocyte osmotic fragility was increased, with initial hemolysis in 0.60% saline and complete hemolysis in 0.38% saline.

* Dr. P. J. Cherney, of Abington Memorial Hospital, Abington, Pa., provided the data on Cases 3 and 4.

He was given two units of whole blood, and splenectomy was performed on Oct. 31, 1956. The spleen weighed 320 gm. His postoperative course was benign.

CASE 5.—A 40-year-old Negro man, a maternal uncle of the first patient, has never been ill and was examined as part of the family study. Physical examination revealed a slim dark Negro man who appeared well. The examination was entirely unremarkable except for the spleen, palpable 4.5 cm. below the costal margin. Laboratory studies revealed a hematocrit value of 41%, numerous spherocytes on peripheral blood smear, and an osmotic fragility showing beginning hemolysis in 0.70% saline and complete hemolysis in 0.65% saline. He has been unavailable for further study.

CASE 6.†—A 49-year-old Negro man, a maternal uncle of the first patient, was admitted to another hospital because of intense pruritus of the feet, hands, and face of one day's duration. He had had a similar episode five years previously. The patient also stated that he had had a yellowish discoloration of his eyes since childhood.

On examination he had a typical Negroid appearance, with dark skin and black curly hair. The sclerae were icteric. The spleen was palpable 4 fingerbreadths below the left costal margin, smooth, and nontender. The hemoglobin was 11 gm. per 100 cc., with a red blood cell count of 3,000,000 per cubic millimeter. There were 16% reticulocytes. The total bilirubin was 10 mg. per 100 cc., with a prompt direct Van den Bergh reaction. The erythrocytic osmotic fragility was increased. The white blood cell count, differential count, and cephalin flocculation were normal. Serological test for syphilis was positive. Abdominal x-rays revealed multiple opaque calculi in the right upper quadrant.

† Mrs. Edith Fields, Medical Record Librarian at Provident Hospital of Baltimore, provided the summary of Case 6.

On June 4, 1954, a splenectomy was performed. The postoperative course was uneventful except for a mild transfusion reaction. On the third postoperative day the hemoglobin was 12 gm. The patient was reported to be asymptomatic in December, 1956.

Comment

Family Studies.—The presumptive diagnosis of congenital spherocytosis in the first patient led us to the examination of as many relatives as were available. Figure 2 diagrams this pedigree. Table 2 summarizes the clinical and hematological data compiled from this family. This family study confirmed our presumptive diagnosis and revealed Patients 2 to 6.

The siblings of the first patient are worth special comment. On March 1, 1947, his mother (Case 2) gave birth to dizygotic twins at another hospital. The first born, a girl, had a history of normal growth and development and is asymptomatic at present. Her physical examination is entirely normal. Her hemoglobin is 12.5 gm., and osmotic fragility studies are normal. The second twin, a boy, did well until the age of 5 days, when he became cyanotic and icteric, refused his formula, developed a purulent umbilical discharge, and died. Autopsy revealed a diagnosis of sepsis and omphalitis. No bacteriological studies were made. The third sibling, a boy, seemed completely normal until 6 months of age, when he developed pneumonia and stiff neck and

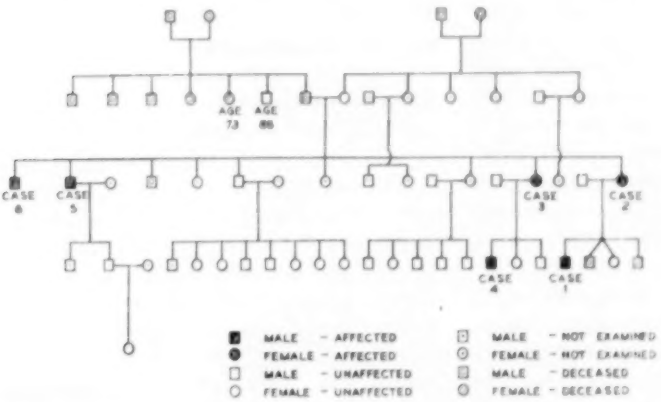

Fig. 2.—Hereditary spherocytosis in a Negro family—genealogical data.

TABLE 2.—*Clinical and Hematological Data of a Negro Family, Six Members with Hereditary Spherocytosis* *

	Icterus	Palpable Spleen	Hb., Gm./100 Cc.	Hematocrit Value, %	Spherocytes	Osmotic Fragility	
						Beginning	Complete
Case 1	0	+	6.4	17	++	0.60	0.50
Case 2 (mother of Case 1)	±	+		27	+++		0.70
Father of Case 1	0	0		40	0	0.45	0.40
Sister of Case 1	0	0	12		0	0.50	0.40
Case 3 (maternal aunt of Case 1)	0	+	11.6		++	I	
Case 4 (son of Case 3)	0	+	5.7		++	0.60	0.38
Father & 2 sibs of Case 4	0	0	N		0	N	N
Case 5 (maternal uncle of Case 1)	0	+		41	++	0.70	0.30
Son of Case 5	0	0		47	0	0.45	0.30
Granddaughter of Case 5	0	0					
Case 6 (maternal uncle of Case 1)	±	+	11	28	++	I	
Maternal uncle	0	0	14	41	0	0.45	0.30
Maternal uncle	0	0	45.5		0	0.60	0.30
12 maternal 1st cousins	0	0					
Maternal grandmother	0	0	13.5		0	0.45	0.40
Maternal great uncle	0	0		42	0	0.45	0.30
Maternal great aunt	0	?					
Maternal grandfather	?	0	12				

* N, normal; I, increased; +, present; 0, absent.

died at home. The father of the first patient was entirely normal.

The maternal grandmother had a physical examination, hematocrit determination, peripheral smear, and osmotic fragility test, but nothing was found to suggest hereditary spherocytosis. The maternal grandfather died at the age of 65 years, but according to his daughter he "had yellow eyes all the time." This was neither confirmed nor denied by his record at another hospital, where he was admitted on two occasions because of a prolapsed hernia. On his first admission, at the age of 58 years, he displayed no recorded physical abnormalities other than a right inguinal hernia. His red blood cell count was 4,110,000 per cubic millimeter, with a hemoglobin value of 12 gm. At the age of 59 years he was readmitted for repair of a strangulated hernia. Partial iliectomy for gangrene was carried out, with no mention of an abnormal spleen. On this occasion his red blood cell count was 3,610,000 per cubic millimeter, with a hemoglobin value of 12.5 gm. per 100 cc.

The maternal great uncle, aged 85 years, was normal on complete examination. The maternal great aunt, aged 56 years, refused examination by us, but her family physician states that she has never had either splenomegaly or icterus. There were four other siblings of the maternal grandfather, but all have died, and no pertinent history is available. The maternal grandmother has four sisters, all living, but they refused study; however, the maternal grandmother states that none of them has ever had any symptoms or signs of anemia, gallbladder disease, or icterus.

The patient's mother (Case 2) had eight siblings, five male and three female. Only five were available for study. Three of these were found to be affected (Cases 3, 5, and 6). The other two were normal, as were all of their children. The husband and two other children of Case 3 were clinically and hematologically normal. The son and granddaughter of Case 5 were clinically normal.

Review of Literature.—The first recorded instance of this disease in a Negro family was reported by Culbreath [8] in 1937. He had two cases of identical twin boys but was unable to complete a family study. Wintrobe,[9] in his textbook (1942), states that this is a rare entity in the Negro race and cites one case in a Negro woman of obviously mixed blood.

There are two questionable cases recorded. Smith and Drake,[10] in 1944, performed splenectomy in a case of hemolytic anemia in a 22-year-old man who had been taking sulfonamides. A symptomatic cure resulted, but the possibility of an acquired process was not ruled out, and no family studies were done. The second case, reported by Merskey [11] in 1946, in an African native, was complicated by concomitant

malaria. Again, no family studies were done.

Scherer and Cecil [12] described a case in a female in 1945, and in 1946 Stragnell and Smith [13] reported a family of five sibs, three of whom were affected. In 1947 three more sibs, all females, were described by Goodman and Cates,[14] and a relative of Scherer's case was presented by McCormack [15] in 1948. Three further cases were described by Churg and Rosenbaum [16] in 1949.

Butterworth,[17] in an excellent review, described in detail two affected patients with five asymptomatic relatives. The latter were considered to be "carriers" rather than to have actual examples of the disease. Irby,[18] in 1953, reported two brothers, with no family studies. Smith and Conley [19] cited a case of sickle-cell trait and hereditary spherocytosis in a Negro in 1954.

Esmond,[20] in 1955, reported three cases, all the children of the earlier case cited by Wintrobe in his textbook in 1942. A rather interesting case was recently published by de Torregrosa.[21] This was a 5-year-old white girl with sickle-cell trait and hereditary spherocytosis. The family (one member a newborn) was of questionable Negroid ancestry.

Shapiro,[22] in discussing two cases in 1957, was not impressed with the rarity of this condition in the Negro race. He has had four cases in the previous three years but does not state in how many families these have occurred.

Conley [23] has studied six cases in three families, one of which was the case cited by Wintrobe. Wurzel [24] has a family of three cases, a mother and two sons, the youngest now being 13 months of age.

Excluding the cases of Smith and Drake,[10] Merskey,[11] de Torregrosa,[21] and the four noncategorized cases of Shapiro,[22] there are now 42 cases of bona fide examples of hereditary spherocytosis occurring in the Negro race. This does include the case of Wintrobe and her family, although of mixed blood.

Of these 42 cases, 17 are male and 25 are female. However, these cases represent only 13 families, making this a rare condition in the Negro race. The age of onset of symptoms ranges from the newborn period [18] to middle age, with the highest frequency of onset of clinical symptoms in the second and third decades.

The previously reported frequency of this disease in the Negro female [14,17] is not so evident when a larger series of cases is analysed. The incidence of the disease is 40% in males and 60% in females, probably an insignificant difference.

"Aplastic" or Aregenerative Phase.—The appearance of an aregenerative crisis in congenital spherocytosis was first described by Owren [25] in 1947. Although Dameshek and Bloom [26] believe this "crisis" to be associated with a hypersplenic mechanism causing bone marrow maturation arrest, recent studies [27] of this type of crisis in sickle-cell disease emphasize the onset of an aregenerative phase without evidence of bone marrow maturation arrest.

The very low reticulocyte count on admission in Case 1 with subsequent rapid rise, with a concomitant rise of the hematocrit value, suggests very strongly that he was in an aregenerative phase on admission to the hospital. Unfortunately, bone marrow studies were not done to support this contention. Although this aregenerative phase often involves all three major marrow elements, this is not invariably true. With the constant hemolysis exhibited by these patients, with resultant shortening of the half-life of the red blood cells, any reduction in the rate of formation of erythrocytes can rapidly lead to a severe degree of anemia. Further evidence of the suggested aregenerative phase in Case 1 is the absence of icterus and the low serum bilirubin value. The decreased red blood cell production diminishes the red blood cell mass available for hemolysis, thus preventing the clinical appearance of icterus. The transient phase of decreased to absent erythropoiesis oc-

curring in congenital spherocytosis in the Negro has also been stressed by Esmond.[20]

It can readily be seen that there is close resemblance between this disease and homozygous sickle-cell disease. Examination of the peripheral blood smear, appropriate osmotic fragility tests, hemoglobin electrophoresis, and family studies should serve to differentiate the two conditions.

Genetic Considerations

The detailed study of 26 European families, comprising 183 members, by Race,[31] in 1942, confirmed the existence of a dominant inheritance of this disease in the white race.

Whether such a hereditary mechanism is operative in the Negro race remains to be proved. If the cases in the Negro race are due purely to an admixture with white blood, with the introduction of the abnormal gene, then we should expect the same mechanism to operate in the Negro race. However, Churg and Rosenbaum [16] mention that several cases in the literature (and including ours) appear to be of as pure Negro stock as exists in this country, although the possibility of preexisting admixture cannot be completely eliminated in any single case.

Butterworth et al.[17] have recently suggested that "multiple [genetic] factors" exist in the Negro cases, which determine the clinical picture produced. Thus those patients with increased red blood cell fragility and spherocytosis but in good clinical health and without overt clinical symptoms are classed by him not as having a mild form of the disease but rather as "carriers." This so-called carrier state he believes to be due to an allelomorph.

The genetic data in this Negro family strongly suggest that the disease is inherited as a dominant in the Negro. We do not believe that there is any justification to classify Cases 3 and 5 as carriers simply because they were asymptomatic and in good health at the time of examination. Rather, they are excellent examples of the spectrum of clinical severity that this disease can manifest. Such a variation in clinical symptomatology is well recognized in the white race.[9,29]

The occurrence of the disease in two sisters (Cases 2 and 3), both with affected children (Cases 1 and 4) and nonaffected husbands, is most probably explained as an example of dominant inheritance. The findings in previously reported Negro families [8,20] are not inconsistent with this hereditary mechanism.

The possibility of a recessive inheritance in such a combination of cases as Cases 1 to 4 is extremely remote. As previously stated, the sex predominance is not as marked as was formerly thought, and so sex-linked inheritance probably does not occur.

Summary

The occurrence of hereditary spherocytosis is rare in the Negro race. Including the 6 cases herein reported, 42 cases in Negroes have been reported. These have occurred in only 13 different family trees. At least one of these families was of obviously mixed blood.

The sex incidence is not as predominantly female as has been previously reported. In these 42 cases the ratio is 3:2, female to male.

The disease varies widely in clinical severity. It may become manifest in the newborn period or be so mild as never to be diagnosed during life.

The available data strongly suggest that the mode of inheritance is similar in the Negro and white races, that is, as a simple dominant. The cases previously classified as "carriers" are more likely examples of the mild form of the disease rather than cases of a clinical variant due to an allelic gene.

The occurrence of a red blood cell aregenerative phase is not uncommon in this disease. In the Negro this must be differentiated by appropriate laboratory and family studies from the similar "crisis" which occurs in homozygous sickle-cell disease.

Splenectomy remains the most adequate treatment for symptomatic cases.

The Johns Hopkins Hospital (5).

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Congenital Hemolytic Anemia in the Newborn

Relationship to Kernicterus

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The natural history of congenital hemolytic jaundice (congenital hemolytic anemia, hereditary spherocytosis) is well known in the medical literature. A number of reviews have pointed out the usual features of this disease, which is characterized clinically by a positive family history, dominant mode of inheritance, recurrent crises, splenomegaly, secondary cholelithiasis, microspherocytes in the blood smear, increased RBC osmotic fragility in hypotonic saline, and effective treatment by means of splenectomy.[1-5]

The age of onset of clinical symptoms has been variable but is stated to be most commonly in childhood or in young adulthood.[4] There are a number of reports concerning symptoms in infancy, the cases usually attracting attention because of anemia.[6-12] The often-quoted maxim, "children, and especially infants, with this disease are more sick than jaundiced," reflects the concepts regarding the usual findings in this age group.[6]

Debré[7] stated that jaundice never shows during the first years, although Tileston,[1] in his early review, suggested that jaundice might be present from birth. Few descriptions are available concerning initial manifestations of hereditary spherocytosis in the newborn infant during the first hours or days of life. In his book on hemolytic anemias, Dacie[4] terms the early onset of jaundice due to hereditary spherocytosis as exceptional in the newborn. In an early report, Dawson[3] referred to three infants who were "born with jaundice." Newns[13] reviewed 20 cases of hereditary spherocytosis from the Hospital for Sick Children, Great Ormond Street, and pointed out that jaundice during the first few days of life was recorded in the histories of five patients, and he suggested that this disease might simulate erythroblastosis fetalis due to blood-group incompatability. Bernard et al.[14] described two cases with a history of jaundice from birth, which lasted to age 2 months in one and to 18 months of age in the other. Both children were said to be retarded, but no mention was made of kernicterus. In a report from St. Christopher's Hospital, Philadelphia,[15] siblings were described who showed abnormal jaundice in the first few hours of life which was considered due to hereditary spherocytosis. One of these infants had clinical kernicterus; the other was treated with exchange transfusion and splenectomy, with good results. Betke[16] has recently described a newborn infant with hereditary spherocytosis, in whom blood-group incompatability was excluded, who developed marked jaundice with neurologic signs "pointing to developing kernicterus" on the 4th day of life; the infant was treated with exchange transfusion.

It is the purpose of this paper to report in detail the first manifestations of hereditary spherocytosis in four newborn infants and to make certain inferences concerning similar signs in other patients in this age period in whom neonatal jaundice may have been the first sign of this disease. In addition, methods of diagnosis and present concepts concerning treatment of congenital hemolytic jaundice in the newborn will be reviewed.

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Report of Cases

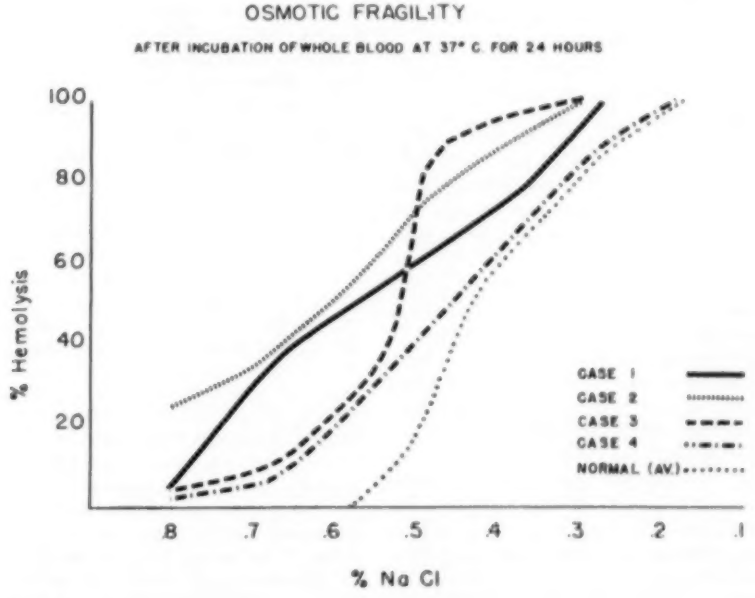
CASE 1.—The patient, admitted Dec. 8, 1955, was referred to the Children's Medical Center at the age of 45 hours because of marked jaundice, first noted at 36 hours of age. He was the product of an uneventful second pregnancy in a 24-year-old mother with known hereditary spherocytosis, who had been treated effectively by splenectomy at the age of 14. The patient's 3-year-old sibling had not been jaundiced in the neonatal period and had never been anemic.

Physical examination revealed an active male infant who was very jaundiced. The weight was 7 lb. 9 oz. (3430 gm.). The liver was not enlarged; the spleen was not palpable. There were no other abnormal physical findings.

Laboratory Findings on Admission.—The hemoglobin concentration was 11.7 gm/100 ml., with an erythrocyte count of 5,780,000 per cubic millimeter and reticulocytes 13.7%. The leukocyte count was 11,700 per cubic millimeter, and the platelet count was 247,000 per cubic millimeter. Many spherocytes were seen in the blood smear. The serum bilirubin was 1.2 mg/100 ml. direct-reacting and 18.8 mg/100 ml. indirect-reacting. The mother's blood group was AB, Rh-negative. No antibodies were present in her serum. The father's blood group was O, Rh-negative. The patient's blood group was B, Rh-negative and the direct Coombs test was negative.

Course.—On the basis of the above findings a diagnosis of hereditary spherocytosis was made. During the first few hours in the hospital, serial serum bilirubin determinations showed a rising level to a maximum of 0.7 mg/100 ml. direct-reacting and 23.8 mg/100 ml. indirect-reacting at the age of 62 hours. Because of the fear of kernicterus from so high a level, an exchange-transfusion was performed at 65 hours of age. By 75 hours of age the serum bilirubin had again increased to 21.3 mg/100 ml. indirect-reacting. It remained at this level for the next 12 hours, then decreased to 14 mg/100 ml. indirect-reacting at the age of 100 hours. The subsequent clinical course was uneventful. He was discharged home at the age of 6 days, at which time the hemoglobin was 9.6 gm/100 ml., with reticulocytes 9.4%, and serum bilirubin was 0.2 mg/100 ml. direct-reacting and 3.3 mg/100 ml. indirect-reacting. He has been followed in the Hematology Clinic at frequent intervals and has remained in a compensated state, with hemoglobin concentration ranging between 9 and 12 gm/100 ml. up to the time of his last visit, at the age of 18 months. There has been no further evidence of jaundice. The spleen tip has been inconstantly palpable. Incubated osmotic fragility tests done at the age of 3 months showed beginning hemolysis in 0.80% saline and complete hemolysis in 0.28% saline, as depicted in the Figure. The patient's mother also showed characteristically abnormal RBC osmotic fragility, while the father and the older sibling showed normal patterns.

CASE 2.—The patient, admitted Sept. 28, 1956, was referred to the Children's Medical Center at



the age of 36 hours because of increasing bilirubinemia. He was the product of an uneventful third pregnancy. The father was known to have hereditary spherocytosis, as his father and grandmother had also had before him. The patient's father had been splenectomized at 8 years of age. The patient's older sibling had never had jaundice or anemia. The next sibling, aged 4 years, had exhibited jaundice in the newborn period and was subsequently proven to have the familial disease, which was treated by removal of the spleen at the age of 5 months. Because of this, the patient was followed expectantly at the referring hospital, where a serum bilirubin level of 12 mg/100 ml. was found at the age of 14 hours. This increased to 25 mg/100 ml. by 32 hours of age, at which time the infant was transferred to the Children's Medical Center.

Physical examination was striking because of intense jaundice. The liver edge was 2.5 cm. below the costal margin. The spleen tip was palpable. The weight was 7 lb. 1 oz. (3203 gm.). There were no other abnormal physical findings.

Laboratory Findings on Admission.—The hemoglobin concentration was 17.3 gm/100 ml. Reticulocytes were 9.3%. Many spherocytes were seen in the blood smear. The serum bilirubin was 0.6 mg/100 ml. direct-reacting and 19.2 mg/100 ml. indirect-reacting. The mother's blood group was O, Rh-positive and the father's blood group was B, Rh-positive. The patient's blood group was O, Rh-positive, and the direct Coombs test was negative. Incubated osmotic fragility tests on blood drawn at the age of 39 hours showed beginning hemolysis in 0.80% saline and complete hemolysis in 0.30% saline, as shown in the Figure.

Course.—An exchange transfusion was performed at 39 hours of age, with decrease in serum bilirubin thereafter to 0.7 mg/100 ml. direct-reacting and 9.5 mg/100 ml. indirect-reading. This was followed by a gradual increase in bilirubin level to 21.2 mg/100 ml. indirect-reacting at 58 hours of age. At this time, a second exchange transfusion was done. After the transfusion there was a satisfactory decrease in bilirubin, but later there was another rise, to 1.1 mg/100 ml. direct-reacting and 17.9 mg/100 ml. indirect-reacting, at 67 hours of age, followed by a definite fall to 12.7 mg/100 ml. indirect-reacting at 86 hours of age. He did well during the remainder of his hospital stay. At the age of 7 days he was discharged home to be followed by his family pediatrician. At the time of discharge the hemoglobin was 13.6 gm/100 ml., reticulocytes were 0.4%, and serum bilirubin was 0.7 mg/100 ml. direct-reacting and 10.7 mg/100 ml. indirect-reacting. At 8 months of age he continues to do well, with hemoglobin 13 to 14 gm/100 ml.

CASE 3.—A boy was admitted to the Children's Medical Center on Nov. 30, 1956, at 76 hours of age, because of increasing jaundice. He was the product of an uneventful pregnancy, with delivery by elective cesarean section at term because of previous section for placenta previa. The mother had been found to have hereditary spherocytosis with cholelithiasis, which was treated by splenectomy and cholecystectomy, seven years previously. An 11-year-old sibling had no jaundice in the neonatal period and had always been well. The other sibling, 7 years old, had shown jaundice at birth which persisted during the first two weeks of life, but he had subsequently been regarded as normal. The patient was noted to have become jaundiced during the first 24 hours of life. In the local hospital, at 50 hours of age, he was found to have a bilirubin level of 20 mg/100 ml., with hemoglobin 16.8 gm/100 ml., and he was referred to us for further care.

Physical examination revealed a markedly icteric hyperactive infant without overt signs of kernicterus. The weight was 7 lb. 13 oz. (3487 gm.). The liver edge was felt 2 cm. below the costal margin; the spleen was not palpable. There were no other abnormal findings.

Laboratory Findings on Admission.—The hemoglobin concentration was 14.0 gm/100 ml., with an erythrocyte count of 4,490,000 per cubic millimeter and reticulocytes 11.8%. The leukocyte count was 15,700 per cubic millimeter, and platelets were 295,000 per cubic millimeter. There were many spherocytes in the blood smear. The serum bilirubin was 1.4 mg/100 ml. direct-reacting and 20.0 mg/100 ml. indirect-reacting. The mother's blood group was A, Rh-positive; there were no abnormal antibodies demonstrable in her serum. The patient's blood group was A, Rh-positive; the direct Coombs test was negative. Incubated osmotic fragility tests, from blood drawn at the age of 81 hours, showed initial hemolysis in 0.80% saline and complete hemolysis in 0.30% saline (Fig.).

Course.—Because of the hyperbilirubinemia and the potential danger of kernicterus, an exchange transfusion was carried out at age 81 hours. There was a decrease in bilirubin to 7.2 mg/100 ml. indirect-reacting after blood exchange. At the age of 96 hours the bilirubin level was 11.6 mg/100 ml. indirect-reacting, and by 120 hours this had decreased to 9.4 mg/100 ml. direct-reacting and 4.6 mg/100 ml. indirect-reacting. He was discharged at the age of 8 days, at which time there were no abnormal physical findings. At the time of discharge the hemoglobin was 11.4 gm/100 ml. and reticulocytes were 0.6%. On follow-up visits, he showed a gradual increase in anemia to a low point—at the age of 2 months, of 5.7 gm/100 ml. of hemoglobin and 5.2% reticulocytes. He was

therefore given a packed red blood cell transfusion. He has subsequently done well, with hemoglobin levels stable at approximately 7 to 8 gm/100 ml. and with continued reticulocytosis to the time of his last visit, at the age of 6 months.

Subsequent fragility tests on this family showed that the patient's 7-year-old sibling, mentioned above as having had abnormal jaundice in the neonatal period, showed markedly increased RBC fragility as well as splenomegaly, spherocytosis, reticulocytosis, and mild anemia. The mother's blood also showed the characteristically abnormal osmotic fragility, while the father and oldest sibling were normal.

Case 4.—A sibling of Case 1, admitted Jan. 4, 1957, was followed expectantly for signs of hereditary spherocytosis by the family physician, who first noted jaundice at the age of 15 hours and referred him to the Children's Medical Center at the age of 30 hours because of increasing jaundice.

Physical examination showed evidence of marked icterus in an active newborn infant. The spleen was not palpable; the liver edge was 1.0 cm. below the costal margin. The weight was 7 lb. 8 oz. (3400 gm.). There were no other demonstrable abnormalities.

Laboratory Findings on Admission.—The hemoglobin was 18.8 gm/100 ml. There were many spherocytes in the blood smear. The bilirubin was 0.6 mg/100 ml. direct-reacting and 16.0 mg/100 ml. indirect-reacting. The mother's blood group was AB, Rh-negative; no antibodies were demonstrable in her serum. The father's blood group was O, Rh-negative. The patient's blood group was A, Rh-negative, and the direct Coombs test was negative.

Course.—Serial bilirubin determinations during the next 18 hours showed a progressive rise to 1.0 mg/100 ml. direct-reacting and 22 mg/100 ml. indirect-reacting at 48 hours of age. Therefore, an exchange transfusion was performed, with subsequent decrease to 0.8 mg/100 ml. direct-reacting and 10.4 mg/100 ml. indirect-reacting bilirubin. Eighteen hours later the bilirubin had increased again, to 23.6 mg/100 ml. indirect-reacting, and a second exchange transfusion was done, which lowered the level to 8.2 mg/100 ml. indirect-reacting. The subsequent clinical course was uneventful. The patient was discharged home at the age of 8 days. Incubated osmotic fragility tests done at the age of 3 months showed beginning hemolysis in 0.80% saline and complete hemolysis in 0.18% saline, as shown in the Figure. Follow-up visits to the Hematology Clinic have shown adequate progress to the present age, of 5 months. He remains fairly well compensated to his hemolytic process, with hemoglobin levels between 7.9 and 9.3 gm/100 ml. and with a persistent reticulocytosis of approximately 10%. There has been no further jaundice. The spleen tip is now palpable.

Diagnosis

As described in this group of newborn infants, hereditary spherocytosis may be the cause of jaundice appearing during the first 36 hours of life. This is a relatively rare cause of jaundice in comparison with the commonest cause at this age—erythroblastosis fetalis secondary to blood group incompatibility. The history of hereditary spherocytosis in a parent is the fundamental point suggesting the diagnosis in an infant presenting with abnormal jaundice during the first few days of life where there is no blood group incompatibility between mother and infant. A history of early neonatal jaundice in a sibling may also be suggestive. The presence of many microspherocytes on blood smear and abnormal erythrocyte osmotic fragility studies, especially of incubated blood, are confirmatory features.

Hereditary spherocytosis in the newborn shares many common features with jaundice

Summary of Pertinent Diagnostic Features

History of hereditary spherocytosis	Case 1 Mother	Case 2 Father, sibling, grandfather, great-grandmother	Case 3 Mother	Case 4 Mother, sibling
History of abnormal neonatal jaundice in sibling	No	Yes	Yes	Yes
Age when onset of jaundice was noted, hr.	36	14	First 24	15
Age at admission to CMC, hr.	48	36	76	30
Bilirubin on admission, mg./100 ml.				
Direct-reacting	1.2	0.6	1.4	0.6
Indirect-reacting	18.8	19.2	20.0	16.0
Hb. on admission, gm./100 ml.	11.7	17.3	14.0	18.8
Palpable spleen	No	Tip	No	No
Spherocytes on smear	Many	Many	Many	Many
Abnormal osmotic fragility RBC	Yes	Yes	Yes	Yes
Blood group of mother	AB, Rh-neg.	O, Rh-pos.	A, Rh-pos.	AB, Rh-neg.
Blood group of patient	B, Rh-neg.	O, Rh-pos.	A, Rh-pos.	A, Rh-neg.

due to ABO incompatibility. Here the initial clinical course may be very similar, with spherocytosis on smear, some increased osmotic fragility of erythrocytes, mild anemia, and little or no splenomegaly.[17-19] There may also be a history of abnormal jaundice in a sibling. At present we have no laboratory test for diagnosis of ABO hemolytic disease, comparable to the Coombs test for Rh incompatibility. A marked elevation in maternal anti-A or anti-B titer may be suggestive but is not diagnostic.[18] The finding of free antibody in the infant's serum of the same type as the infant's major blood group may lend further support to the clinical diagnosis.[19] At present the finding of spherocytosis in a parent is the best diagnostically differentiating point between these two diseases. Although rare cases of supposed hereditary spherocytosis have been reported in which the parents were apparently normal, this is exceptional.[4,5] In the absence of a family history, the subsequent course should clarify the diagnosis in that evidences of hereditary spherocytosis are permanent, while manifestations of hemolytic disease due to ABO incompatibility are confined to the neonatal period.

In addition to hemolytic disease due to blood group incompatibility, there are many other processes which may be associated with jaundice in the newborn, including physiologic jaundice, congenital nonspherocytic hemolytic anemia, cytomegalic inclusion disease, toxoplasmosis, syphilis, sepsis, and neonatal hepatitis.[20] Generally these states may be differentiated by the absence of a family history of hereditary spherocytosis as well as other features which are associated with the particular disease.

Treatment

Treatment by exchange transfusion was given these infants because of the known correlation between hyperbilirubinemia and kernicterus in the newborn.[21,22] We have no known example of kernicterus in our group of patients, although a case of kernicterus probably due to hereditary spherocytosis has been described.[15] In addition to controlling hyperbilirubinemia, exchange transfusion provides normal red blood cells which will exhibit a normal life span [12,23] and thereby protect the infant from the potential dangers of a severe hemolytic or hypoplastic crisis [20] during the first few weeks of life.

Definitive treatment by splenectomy has been deferred in these four infants thus far. We have elected to pursue a course of careful follow-up, during which time this particular group has done well. Despite evidence of increased hemolysis they have been able to compensate by increased blood production, and their anemia has remained mild. We have encountered other infants in whom the level of adjustment between hemolysis and blood production was not satisfactory, with resultant severe anemia necessitating repeated transfusions and splenectomy. The following case * is an example of this situation.

A girl was admitted to the Children's Medical Center at the age of 5 weeks because of congenital hemolytic anemia. At the local hospital jaundice had been noted during the first 24 hours of life and had remained a prominent sign during the first 3 days. At the age of one week the hemoglobin was recorded as 11.3 gm/100 ml. Osmotic fragility tests were reported as showing initial hemolysis in 0.70% saline, with complete hemolysis in 0.40% saline, as compared with a normal control which showed initial hemolysis in 0.45% saline and complete hemolysis in 0.3% saline. At the age of 2 weeks the hemoglobin was 5.6 gm/100 ml. and the patient was given a blood transfusion. Because of progressive anemia she was subsequently referred to the Children's Medical Center, where her sibling had been previously treated by splenectomy at the age of one year for the same disease. The father, in whom the diagnosis of hereditary spherocytosis had been clearly established, had also been effectively treated by splenectomy.

Physical examination showed a pale irritable infant. The spleen was felt 1 cm. below the costal margin. There were no other abnormal physical findings.

* Dr. Ronald L. Denton, of Montreal, who cared for this patient during the neonatal period, provided us with details concerning the early history of this patient.

Laboratory Findings on Admission.—The hemoglobin was 5.7 gm/100 ml. The erythrocyte count was 2,100,000 per cubic millimeter, and reticulocytes were 7.5%. Spherocytes were present in the blood smear.

Course.—The infant was treated with transfusion of 75 ml. of packed red blood cells and was discharged to be followed. There was a progressive increase in anemia over the next three weeks, which resulted in a decision to perform splenectomy at the age of 2 months. This was accomplished uneventfully, and the infant has subsequently exhibited a normal course.

A number of reports have suggested an association between splenectomy and severe infection in infancy and childhood.[11,24,25] In our experience this has been unusual when splenectomy has been performed for hereditary spherocytosis.[25] However, we have only considered splenectomy on the basis of active manifestations of the disease in the individual infant with severe recurrent anemia due to hemolytic or hypoplastic episodes. We have not advised splenectomy on elective grounds in an infant whose disease is well compensated.

Comment

It is apparent from these cases that jaundice during the first 36 hours of life may be the earliest manifestation of hereditary spherocytosis. In an effort to obtain some idea of the frequency of jaundice in the newborn as the first sign of his disease, we have reviewed records of such patients at the Children's Medical Center during the past 10 years. This encompassed 52 patients, proved to have hereditary spherocytosis, who were admitted at 1 week to 10 years of age. Information concerning events during the first few days of life varied according to the accuracy of the admission history, which in almost all instances was derived from parental recall rather than from direct information from the physician in attendance during the newborn period. Jaundice in the first few days of life was recorded in 23 of the 43 histories in which adequate detail was available. In four instances an etiologic label had been suggested: sepsis in one, A-O blood-group incompatibility in one, and physiologic jaundice in two. It is impossible to define the basis for jaundice in any one of these cases by this sort of retrospective review. However, this does suggest that jaundice in the first few days of life is a commonly recorded feature in the history of a child in whom the diagnosis of hereditary spherocytosis is made at a later age.

We believe that the offspring of a parent with hereditary spherocytosis should be observed carefully in the newborn period for evidence of abnormal jaundice in a manner similar to the way in which an infant of a sensitized Rh-negative mother is regarded. The appearance of abnormal jaundice in the newborn in such cases suggests that the infant may also have hereditary spherocytosis. If the infant with hereditary spherocytosis develops severe jaundice in the first few days, he should be treated with exchange transfusion just as promptly as the erythroblastotic infant, since hyperbilirubinemia in such an infant may be just as dangerous to the nervous system, i. e., may produce kernicterus as in erythroblastosis. Likewise, repeat exchange transfusion for hyperbilirubinemia is equally important when the level of indirect-reacting bilirubin reaches over 20 to 25 mg/100 ml.

With regard to splenectomy, it is generally accepted that the operation is curative for patients with hereditary spherocytosis. However, it should only be advised in young infants because of recurrent severe anemia either due to uncompensated hemolytic crises or hypoplastic episodes. In the face of either of these events, it is our opinion that it is more hazardous to leave the spleen in than to remove it and face the rather small risk of overwhelming sepsis, particularly due to pneumococci, in a splenectomized infant.

Summary

Hereditary spherocytosis in the newborn may be initially manifested by the appearance of jaundice in the first 36 hours of life, as exemplified by four recent cases at the

Children's Medical Center. Methods of confirming the diagnosis are described. The importance of exchange transfusion in treatment of hyperbilirubinemia in order to prevent kernicterus is emphasized. Definitive treatment by splenectomy may be delayed in infancy, depending on the severity of the manifestations in the individual infant. With careful consideration of these factors, the prognosis in an infant with this disease is excellent.

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Addendum

All these patients were seen in September and October, 1957, at which time they were aged 23, 13, 11, and 10 months, respectively. They were all in good health and had had no serious infections or crises of hemolysis.

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Studies on Cyclic Neutropenia

A Clinical and Experimental Investigation

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Cyclic neutropenia is a rare disease characterized by the regular disappearance of neutrohils from the circulating blood at approximately 21-day intervals. The neutropenic phase of the cycle is generally associated with clinical manifestations of fever, malaise, and the appearance of ulcers in the oral mucous membranes. Other symptoms, which are not common to all the patients, include intermittent arthralgia, abdominal pain, sore throat, lymphadenitis, headache, ischiorectal infections, mental depression, conjunctivitis, and cutaneous ulcers. To date only 23 well-documented cases have been reported in the literature. A summary of these cases is presented in Table 1.

The neutrophils in the peripheral blood regularly fall to extremely low levels and often disappear for several days during each cycle. Between attacks the neutrophils may rise to nearly normal levels, but in most of the reported cases the neutrophil count is always somewhat lower than normal and seldom exceeds 50% of the differential count. In some cases the highest count reached does not exceed 20% of the circulating leukocytes. The total white blood cell count stays rather constant in the low normal range throughout the cycle in some cases and the deficit of neutrophils is made up by the occurrence of a compensatory monocytosis. In other cases the WBC count falls to low levels during the neutropenic phase and rises to normal between attacks. The clinical symptoms usually appear one to three days prior to the disappearance of the neutrophils from the circulation.

Bone marrow studies have been done on most of the reported cases, with similar results. In all instances the marrows have been hypoplastic. In a few cases [7,12,19] where an isolated marrow sample was taken during the neutropenic phase, an abundance of early granulocytic forms was found and few or no mature forms were present. This picture has been referred to as a "maturation arrest." However, in cases where serial samplings of the bone marrow were studied,[8,13,14,18] the immature granulocytes disappeared from the marrow prior to the onset of neutropenia and they reappeared in the marrow before neutrophils reappeared in the blood. The evidence thus far presented can be interpreted as support for the concept that the cyclic fluctuations of the circulating neutrophils are but a reflection of cyclic changes taking place in the bone marrow.

Etiology

Attempts to discover the cause of this disease have thus far been futile. The similarity of the cyclic fluctuations to the menstrual cycle have led several workers to investigate hormone excretion. Thompson [3] studied a 25-year-old man who had had cyclic neutropenia from infancy. This patient also had a two-year history of diabetes insipidus which was controlled with vasopressin (Pitressin). The patient's

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TABLE 1.—*Cyclic Neutropenia: Summary of Twenty-Three Cases, Listed in Approximate Order of Report*

Case	Symptoms	Bone Marrow	Age	Sex	Duration	Cycle Length, Days	Hormone Excretion	Treatment	Spleen Removed	Splenectomy Results or Remarks
1 Leale [1] Rutledge [2] Thompson [3]	Fever, stomatitis, lymphadenitis	None	25	M	Since infancy	21	Female type of hormone excretion	Autogenous vaccine	—	The patient's serum decreased the motility of normal leukocytes
2 Sutton [4]	Fever, stomatitis	None	16	M	Since infancy	21	....	....	—	No blood cell counts taken on proven case
3 Doan [5]	Fever, skin ulcers, stomatitis	None	18	F	Not given	21	Unrelated to menses	Pentose nucleotide	+	Symptomatic relief, but continued cycles with a higher WBC level
4 Embleton [6]	Fever, stomatitis, malaise	None	43	F	....	17–19	Unrelated to menses	....	—	
5 Imerslund [7]	Fever, stomatitis, malaise	Many precursors, no mature forms	16	M	Since infancy	21	No abnormality in female hormones	....	—	
6 Barsby [8]	Fever, stomatitis, arthralgia, abdominalgia	Hypoplastic marrow changes before blood	41	F	....	21–28	Questionable relation to menses	Pentose nucleotide, yellow bone marrow, liver, iron, niacin, estrogen	—	
7 Vahlquist [9]	Fever, skin abscess, otitis media	Hypoplastic	4	F	Since age 2 mo.	21	....	Pentose nucleotide	—	
8 Reznikoff [10]	Fever, abdominalgia	Hypoplastic	18	M	Since infancy	21	Female hormones normal, 17-keto. low at onset	Adrenocortical extract ineffective	+	Symptomatic relief, bone marrow count increased, peripheral blood unchanged; patient's serum inhibited rabbit bone marrow cells
9 Barling [11]	Fever, stomatitis, vulvar ulcers when pregnant	None	32	F	Since age 12	21–28	Persistence of ulcers when pregnant	....	—	
10 Löffler [12]	Fever, arthralgia, lymphadenitis	Many precursors, no mature forms	30	M	1 yr.	21	....	....	+	No benefit, spleen showed inflammatory hyperplasia
11 Owren [13]	Fever, stomatitis	Hypoplastic marrow before blood	23	M	5 yr.	20	....	Pentose nucleotide	+	No benefit, spleen enlarged with nonspecific reactive changes
12 Fullerton [14]	Fever, local infection, tonsillitis, malaise	Hypoplastic marrow before blood	62	M	1 yr.	23–28	Normal female hormones and 17-ketosteroids	Diphenhydramine, pyradoxine	+	Symptomatic relief, cycles continued at a higher WBC level, spleen enlarged with hyaline degeneration of central arteries
13 Erf [15]	Fever, stomatitis	Hypoplastic normal diff.	55	F	2 yr.	28	....	....	+	Symptomatic relief, steady fall in WBC count after an initial rise, reticular hyperplasia of spleen; not a recognized case
14 Alt, cited by Reimann [16]	Fever, stomatitis	....	18	M	Since infancy	18–22	....	....	—	
15 Reimann [16]	Fever, stomatitis, arthralgia, lymphadenitis	Hypoplastic	23	M	5 yr.	21	Normal female hormones 17-ketosteroid corticotropin response	Yeast, liver, bone marrow	—	

16 Cobet[17]	Fever, stomatitis, depression	....	25	F	Since infancy	21	Unrelated to menses	....	+	Symptomatic relief, no change in blood picture, normal spleen
17 Borne[18]	Fever, local infection	Hypoplastic marrow before blood	3	F	Since age 5 mo.	21	Estrogen and prolan excret. at onset	Pentose nucleotide, vaccine	—	
18 Rolland[19]	Fever, stomatitis, lymphadenitis	Many precursors, no mature forms	8	F	Since age 7 mo.	21	Normal female hormones	....	—	
19 Sandella[19]	Fever, stomatitis, malaise	Hypoplastic	64	F	1 yr.	20-22	....	....	+	Symptomatic relief, blood cycles lengthened, reticular hyperplasia of spleen, died 16 months later from lymphosarcoma
20 Monteleff[11]	Fever, sore throats	....	2	M	....	....	....	....	—	Cured by tonsillectomy, not a well-documented case of cyclic neutropenia
21 Monto[11]	Fever, stomatitis,	Hypoplastic	77	M	1 yr.	21	....	Corticotropin, dramatic results	+	Symptomatic relief, constant neutropenia followed an initial neutrophilia; sclerosis of central arteries, mild
22 Siguier[11]	Fever, stakerrhea	....	42	M	7 yr.	20	....	Corticotropin, cortisone, ineffective	—	
23 Good[24]	Generalized infections	Failure of maturation	2	M	Since 7 mo.	21	....	....	—	Associated agammaglobulinemia

urinary excretion of estrogen and gonadotropins was similar to the normal menstruating female pattern, and the fluctuations were thought to be in phase with the patient's neutropenia cycles.

Thompson also reported several cases of acute agranulocytosis that had occurred in females at or near the onset of menstruation. Later, Jackson and Merrill[21] studied a 30-year-old woman who had four attacks of agranulocytosis during a two-year period. These attacks all occurred at the onset of menstruation. Stephens and Lawrence[25] also reported the case of a 38-year-old woman who had several attacks of agranulocytosis which occurred with onset of the menses. This patient regularly took aminopyrine for dysmenorrhea. She continued to have attacks of agranulocytosis at irregular intervals after having a bilateral oophorectomy, until all drugs except codeine were discontinued. Probably this was a case of aminopyrine sensitization instead of true cyclic agranulocytosis. Later Plum[26] showed a definite relation between ingestion of aminopyrine and the development of agranulocytosis. He pointed out that the most probable cause for the relation of agranulocytosis to menstruation was the use of aminopyrine for dysmenorrhea.

Barsby[8] studied a 41-year-old woman who had attacks of cyclic neutropenia which seemed to occur in close temporal relationship to menstruation; however, he followed her with serial counts for seven months and could not establish a definite relationship of the neutropenia to the menstrual cycle. Barling[11] described a 32-year-old woman who had cyclic attacks of fever and oral ulcers for 20 years. She developed persistent oral and vulval ulcers during pregnancy. Serial blood cell counts were not done, but scattered counts revealed a neutropenia. The only other case of cyclic neutropenia in which female sex hormones could be implicated was that of a 3-year-old girl reported by Borne.[18] This patient had small amounts of urinary estrogen and gonadotropins at the onset of her attacks.

These hormones were absent from her urine between attacks.

In three cases[5,6,17] of cyclic neutropenia in menstruating women, no relation to the menstrual cycle could be found. Also, there are four reports[7,14,16,19] of cyclic neutropenia occurring in males whose excretion of estrogen and gonadotropins was normal.

Reznikoff[19] studied an 18-year-old youth who had cyclic neutropenia from infancy. He found the patient's 17-ketosteroid excretion decreased with the onset of each cycle. Fullerton[14] reported a 62-year-old man who had no fluctuations in 17-ketosteroid excretion, and a 19-year-old youth studied by Reimann[16] had both normal 17-ketosteroid excretion and normal corticotropin (ACTH) response tests during and between attacks of neutropenia.

Although suggestions of disturbed endocrine function in these patients have appeared sporadically in the literature, the available evidence indicates that no consistent hormonal imbalance has as yet been implicated in these patients.

Wiseman and Doan[27] called attention to the spleen as a factor in neutropenia with their description of splenic neutropenia. This disease may be cured by splenectomy. Doan first tried splenectomy as a cure for cyclic neutropenia in an 18-year-old female patient. Although operation did not stop the cyclic fluctuations in the neutrophil count, the counts remained at higher levels during each phase of the cycle than preoperatively and the patient's symptoms of fever and oral ulcers were less severe after the operation.

Reznikoff's 18-year-old male patient[19] also obtained symptomatic relief from splenectomy. The bone marrow WBC count changed from 1800 per cubic millimeter before the operation to 15,000 per cubic millimeter after the operation; however, there was no change in the fluctuations of neutrophils. The spleen was enlarged and showed severe sclerosis of the central arteries.

Fullerton[14] reported the case of a 62-year-old man, with a one-year history of recurrent infections and neutropenia, who received marked symptomatic relief from splenectomy. The neutrophil count, however, continued to fluctuate regularly, but at higher levels. The spleen was enlarged to four times normal size, and there was hyaline degeneration of the central arteries.

Erf and Fry[15] studied a 55-year-old woman with a two-year history of recurrent fever, oral ulcers, and neutropenia. After splenectomy her total leukocyte count rose rapidly and then began to decline gradually during the two years she was observed. Her symptoms were relieved, but serial WBC counts were not done to see whether cyclic fluctuations were present. The spleen weighed 780 gm. and exhibited reticular hyperplasia.

Cobet[17] studied a young woman who had had cyclic neutropenia since infancy. She received some symptomatic relief from splenectomy without any changes in the blood picture. The spleen was normal.

Sandella[20] reported the case of a 64-year-old woman who after having regular neutropenic cycles for six months was completely relieved of symptoms by splenectomy. She had suffered with fever, stomatitis, and conjunctivitis. The neutrophil fluctuations persisted, but they were less severe and the intervals between cycles were longer following splenectomy. The spleen weighed 670 gm. and showed reticular hyperplasia. Sixteen months later this patient died with extensive lymphosarcoma of the bones, viscera, and lymph nodes. Review of the spleen and of a liver biopsy specimen taken at the operation did not reveal any evidence of lymphosarcoma.

Monto's patient,[22] a 78-year-old man who had cycles of fever and oral ulcerations for one year, was relieved of these symptoms by splenectomy. This patient had a persistent neutropenia, with a level of about 140 cells per cubic millimeter, preoperatively. The operation produced an initial neutrophilia, but the WBC count

gradually dropped to leukopenic levels and the neutropenia returned. The spleen showed a mild degree of sclerosis of the central arteries.

Two patients were not improved by splenectomy. They were a 30-year-old man [12] with a one-year history of regular attacks of arthralgia and neutropenia, whose spleen showed inflammatory hyperplasia, and a 23-year-old man [13] with a five-year history of regular attacks of fever, stomatitis, and neutropenia whose spleen was slightly enlarged and showed nonspecific reactive changes.

In summary, seven of nine patients diagnosed as having cyclic neutropenia who had splenectomy received some degree of symptomatic relief, while two patients were not helped. The basic cyclic fluctuations in the neutrophil count were unchanged in all the patients except one. In several cases the WBC and neutrophil counts remained at higher levels following the operation, but in other cases counts were unchanged. Interestingly enough, some patients were relieved of symptoms in the absence of changes in the blood picture. This observation supports the concept that the neutropenia does not cause the symptoms but rather that both are manifestations of some basic periodic disorder. The removed spleens, with the exception of one, were all enlarged, and they exhibited a variety of pathological lesions. None of the lesions can be correlated with success or failure of the operation. The mechanism by which the spleen might suppress the WBC count or contribute to the symptoms of the disease is not known.

Work by Moeschlin [28] and others [29-34] has shown that chronic neutropenia can be caused by autoantibodies. This followed earlier work by Moeschlin, who submitted evidence indicating that an antibody-antigen reaction is responsible for the aminopyrine type of agranulocytosis. It is not yet clear whether antileukocytic antibodies play a role in cyclic neutropenia. Rutledge [2] found that his patient's serum inhibited the motility of normal leukocytes, and Reznikoff [10] found that serum from his patient inhibited respiration and motility of rabbit bone marrow cells. However, Fullerton [14] transfused plasma from 500 cc. of his patient's blood into a normal recipient on two occasions with no subsequent fall in leukocyte or neutrophil counts. As yet, no one has demonstrated in vitro agglutination of normal leukocytes by serum from a patient with cyclic neutropenia.

An interesting observation by one of us [35] was the presence of cyclic neutropenia in a patient who also had agammaglobulinemia. This patient was studied over a 4-month period, during which he exhibited profound neutropenia every 21 days. His course differed from other cases of cyclic neutropenia in that his life was constantly threatened by overwhelming bacterial infections, and he finally died of pneumonia. In this patient cyclic neutropenia occurred in the absence of circulating antibody, and at a time when the patient was unable to manufacture antibody. Electrophoretic analysis of the serum proteins has not been carried out on any of the other reported cases of cyclic neutropenia; therefore, the possibility that some of the other cases reported were also associated with agammaglobulinemia exists. However, this possibility seems unlikely, since patients with agammaglobulinemia as a rule have been subject to numerous serious bacterial infections, while in most of the reported cases of cyclic neutropenia the relative freedom from serious infection has been worthy of comment.

Recently Reimann [36] has called attention to a group of disorders he prefers to call periodic disease. He includes in this group cases of cyclic neutropenia, abdominalgia, arthralgia, fever, sialorrhea, skin lesions, and other disorders, all of which recur at three-week intervals. He believes that these diseases are all related and all reflect the same basic physiological or pathological cycle.

Therapy

There is no specific therapy for cyclic neutropenia. Pentnucleotide (pentose nucleotides from the ribonucleic acid of yeast), which was shown by Doan[5] to have a stimulating effect on normal bone marrow, has been tried in at least five cases,[5,8,9,13,18] with no benefit. Other blood-building agents, such as liver, iron, nicotinic acid (niacin), yellow bone marrow, pyridoxine, and dried (brewer's) yeast have all been ineffective in influencing the disease. Progesterone, estrogen,[18] and testosterone[16] have also been unsatisfactory. Diphenhydramine (Benadryl) and steroids, agents aimed at suppressing allergic phenomena, have been tried. Diphenhydramine was used by Fullerton[14] and had no effect. Adrenocortical extract was used by Reznikoff,[19] with no effect on the patient's disorder. Monto[22] started his patient on corticotropin when the patient was moribund with pneumonia. The patient made a dramatic clinical recovery, and his bone marrow reverted from a hypoplastic state to hyperplastic condition. Splenectomy was then performed, and the prolonged effect of corticotropin was not studied. Siguier[23] used both corticotropin and cortisone on his patient, with no effect.

Splenectomy, which has already been discussed, is the only means of therapy thus far tested which appears to have any effect on this disease, and even this approach has not consistently altered the cyclic neutropenia.

Consequently, present treatment of this disease depends primarily on supportive measures. Prophylactic antibiotics and vigorous early treatment of infections has been used in these patients to maintain freedom from life-threatening crises due to severe infection. Fortunately, these patients do not appear to be nearly as susceptible to serious infections as are patients with other acute forms of agranulocytosis. It may be that the monocytosis which many of them have during the neutropenic phase of the disease provides protection not available to patients with other forms of agranulocytosis. However, even in the absence of demonstrable infection the symptoms of malaise, oral ulcers, fever, and headache, which occur simultaneously with the agranulocytosis, are so distressing that most of the patients with cyclic neutropenia spend several days each month in bed. Specific therapy in this disease still awaits basic understanding of the underlying cyclic process.

The purpose of this paper is fourfold, namely, to present an additional case of cyclic neutropenia, to describe extensive clinical studies performed in an effort to define this condition more precisely, to report the results of several attempts at treatment of the disease, and, finally, to relate certain observations made in an effort to interpret this "experiment of nature" which should shed light on the role of neutrophils in the body economy.

Report of a Case

A 15-year-old white girl was the product of a normal gestational period and normal delivery. She was well until 5 months of age, when she developed whooping cough. This disease left her with a persistent cough productive of thick white sputum, which lasted until she was 6 years old and then cleared spontaneously. At the age of 6 months she developed generalized impetigo, which persisted for 6 weeks. At one year of age she had a similar eruption. Both of the episodes left permanent scars on the skin that resemble the scars of generalized vaccinia. When 3 years of age she developed a sore on the tip and left side of her tongue. This progressed in size and became a large ulcer which healed after six weeks with much scarring. Because of this illness, a routine blood study, including a total white cell count and differential count, was performed, which revealed a marked decrease in the number of circulating neutrophils. From that time forward the child developed, at intervals, recurrent necrotizing lesions of the oral mucous membranes associated with skin infections, malaise, fever, and anorexia.

The patient was seen at the University of Minnesota Hospitals for the first time when she was 4½ years old, with the chief complaint of recurrent fever and oral ulcers. A white blood cell count and a differential count again revealed neutropenia. An original clinical presumption that the lesions were syphilitic was eliminated when

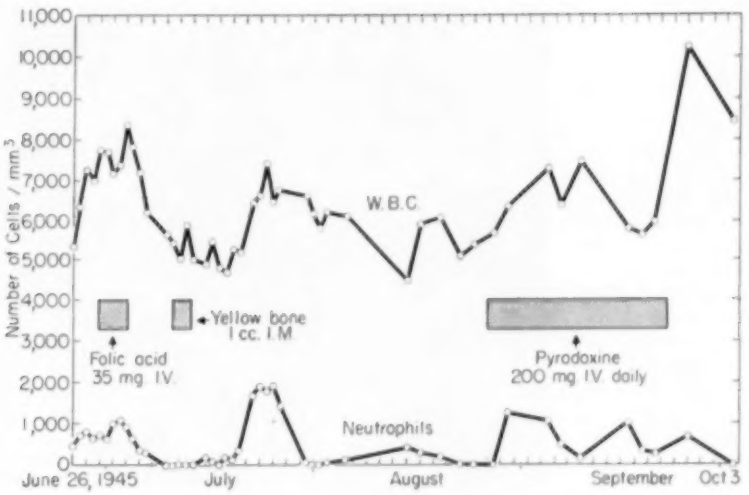


Fig. 1.—Serial neutrophil counts illustrating cyclic neutropenia occurring at approximately 21-day intervals when the patient was 4½ years old. During the early phase of the disease the cylic disappearance of neutrophils was associated with persistent neutropenia and absolute lymphocytosis. During this phase of the disease treatment with folic acid, yellow bone marrow, pyridoxine, and diathermy was tried, but none produced a significant effect on the clinical or laboratory features of the disease.

it was found repeatedly that the Wassermann tests were negative, and dark-field examinations of scrapings from the ulcers revealed no spirochetes. Initially, although the neutropenia was known to fluctuate in severity, it was thought to represent a persistent blood dyscrasia. Bone marrow examination revealed neutrophilic hypoplasia and contained increased numbers of eosinophils and plasma cells. The diagnosis made initially was idiopathic neutropenia or agranulocytosis.

A few weeks following the initial study the patient was readmitted to the University of Minnesota Hospitals for therapeutic trial on folic acid, yellow bone marrow, and pyridoxine. Over a four-month period of study, white blood cell counts and differential counts were made at frequent intervals. The results of this study are summarized in Figure 1. Although it was not appreciated at the time, it is apparent from examination of the Figure in retrospect that cyclic neutropenia was present even at this early date and that the neutrophilic counts revealed fluctuations with approximately a 21-day periodicity. None of the therapeutic agents tried had any effect on the neutropenia.

During the subsequent six years she was admitted to the hospital on 25 occasions. Hospitalization was usually necessary to treat infections which had developed during the periods of neutropenia. The infections included numerous episodes of cellulitis of fingers, toes, and lips; an axillary abscess; otitis media; maxillary abscess; cervical lymphadenitis, and abscessed teeth. In several instances, if the beginning cellulitis was neglected, lymphangitis and septicemia occurred. All of these infections responded promptly to antibiotics, penicillin being the agent most frequently used. With each admission, neutropenia was noted and often ulceration of the oral mucosa was present. The white blood cell count was usually in the normal range, the neutrophils were often 0 or 1%, and a monocytosis as high as 28% was often noted when the neutrophil count was low. The highest neutrophil count recorded during this period was 1740 per cubic millimeters.

During this period, when the child would be presented at the hospital with neutropenia, infection, fever, oral ulcers, and malaise, penicillin would be administered over a period of several days and the entire clinical disease would disappear. It was thought by most of the attending pediatricians that penicillin treatment was responsible for abatement of the infection as well as clearance of the necrotizing processes and improvement of the blood picture. As a matter of fact, penicillin was considered by some to be a specific remedy for her recurrent disease.

On two occasions, when she was 6½ and 7 years old, she was treated with short-wave diathermy directed to the ribs, sternum, and extremities. The rationale for this approach was the success of diathermy in the treatment of hypoplastic anemia reported by Nelson and Aldrich.[47] In the neutropenic disease, however, diathermy was of no

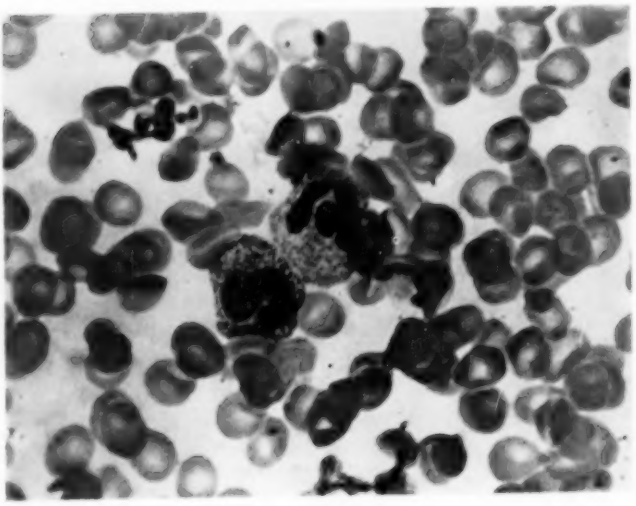


Fig. 2.—In this photomicrograph the "toxic" characteristics of the neutrophils in the peripheral blood are illustrated. This type of cell has always characterized the peripheral smear, even during the periods when the neutrophils were at their highest concentrations in the peripheral blood. Note the large size of the cells and their large basophilic granules; × 1600.

benefit. In 1952, when 11 years old, the patient was reinvestigated; the cyclic nature of her disease was appreciated, and a diagnosis of cyclic neutropenia was established. Since that time, the oral ulcers, malaise, and profound neutropenia have continued to occur at regular intervals, and it has been clearly established that penicillin is of value only in treating the intercurrent infections due to organisms susceptible to this antibiotic and is in no wise a remedy for the cyclic disease. During the last few years the cyclic nature of the neutropenia has become even more apparent because the neutrophil count now reaches higher levels in the neutrophilic episodes than was previously the case. During the neutropenic episodes the patient becomes clinically ill, developing fever, severe malaise, ulceration of the oral and pharyngeal mucosa, and profound neutropenia. The neutrophil count remains at extremely low levels for five to seven days; indeed, for two to five days absolutely no neutrophils can be found in the peripheral blood. When neutrophils are found in the peripheral blood, even during periods when their number approaches normal, they are abnormal morphologically, having the appearance of "toxic neutrophils," with large basophilic granules (Fig. 2). During the periods of remission the total number of neutrophils is usually slightly lower than normal. Almost as though reciprocally related to the neutrophil count, true monocytosis develops during the periods of neutropenia, and as many as 30% of the circulating cells may be monocytes. Eosinophilia is a persistent anomaly of the peripheral blood, and the numbers of eosinophils fluctuate between 10% and 15% of the white cells, without relation to the cyclic changes in neutrophil count. The patient's total white cell count fluctuates between 3000 and 5000 cells per cubic millimeter. The variations in total white cell count bear only a rough relationship to the 21-day neutrophil cycle. The hemoglobin concentration and red cell count have remained normal throughout the entire illness. Figure 3 is a graphic representation of the fluctuating numbers of leukocytes during a six-month period. This graph clearly shows the cyclic fluctuation of the neutrophils and the reciprocal monocytosis which occur with a 21-day periodicity. It is to be emphasized that the periodicity is extraordinarily constant. Literally every 21 days the neutrophils disappear from the blood. Also to be noted on the graph is the striking reciprocal relationship of true monocytosis with the neutropenia.

On several occasions the patient has had infections requiring prolonged antibiotic therapy. In recent years infections with antibiotic-resistant staphylococci have become a serious problem. Recently she had a prolonged attack of otitis media, during which time the cyclic nature of her disease was abolished and the neutropenia was persistent over a two-month period. During this interval she was free of oral ulcers. However, following successful treatment of the otitis media the typical 21-day cycles were once again established and oral ulcers were again a problem every 21 days.

In spite of the frequent severe infections and the intermittent disease which occurs regularly every three weeks, her growth and development have been excellent. Plotted on Figure 4 is a comparison of her growth curve with the mean

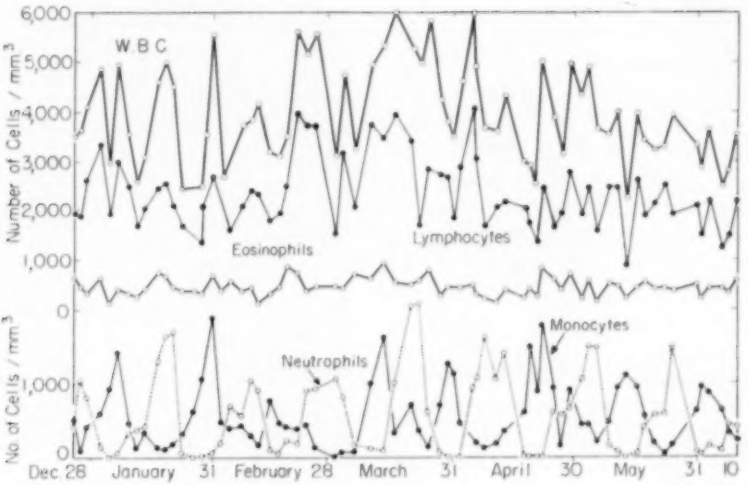


Fig. 3.—The results of serial white blood cell counts and differential counts carried out every two to three days over a six-month period when the patient was 14 years of age. Note the clear-cut cycling of the neutrophil count at 21-day intervals and the reciprocal relationship between the absolute neutrophil count and the absolute number of monocytes during this period. The numbers of lymphocytes, eosinophils, and total white cell count show variations which appear to be independent of the variations in the numbers of neutrophils.

growth curves for normal girls in Iowa.* As may be seen from this record, the patient has always been above average in height and weight and has shown no deviation from her expected progress as a consequence of this illness. This patient, who has had numerous intercurrent infections and periodic illness, in the aggregate occupying almost one-third of her life, represents a striking illustration to support the concept that interference with growth occurs only when infection and disease are truly chronic and not intermittent. Similar observations have been made in patients with agammaglobulinemia.

In addition, her physiological maturation has been normal. She passed through a normal puberty, began menstruating at 12½ years of age, and has had regular menstrual cycles for more than three years. Her breasts are well developed, and she has pubic and axillary hair in a normal female distribution. As may be inferred from Figure 3, the menstrual cycle appears to bear no relationship to the cyclic neutropenia. Figure 5 is a picture of the patient taken when she was 14 years of age. It will be seen that she is a large well-developed girl in spite of her intermittent disease and the recurrent infections that have been her lot.

Attempt to Demonstrate Circulating Antibody Using in Vitro Methods.—Recent work by Dausset,[29] Moeschlin,[28] and others[31,32,34] has shown that neutropenia can be produced by the formation of "auto-antibodies." Indeed, evidence has been presented to indicate that certain forms of human agranulocytosis are due to the existence in the circulation of antibodies directed against white blood cells, and experimental production of isoantibodies has permitted production of experimental neutropenia and leukopenia.[38,39] It was considered possible that such antibodies might be released intermittently into the circulation and account for the cyclic neutropenia observed. Since no search for circulating antileukocyte antibodies has been recorded in cyclic neutropenia and since the finding of slight plasmacytosis and hypergammaglobulinemia in our patient was compatible with this hypothesis, an exhaustive attempt has been made to demonstrate antineutrophilic anti-

*The standard curves of height and weight growth used as bases for comparison were prepared from data compiled by the Iowa Child Welfare Research Station and copyrighted in 1943 by the State University of Iowa.

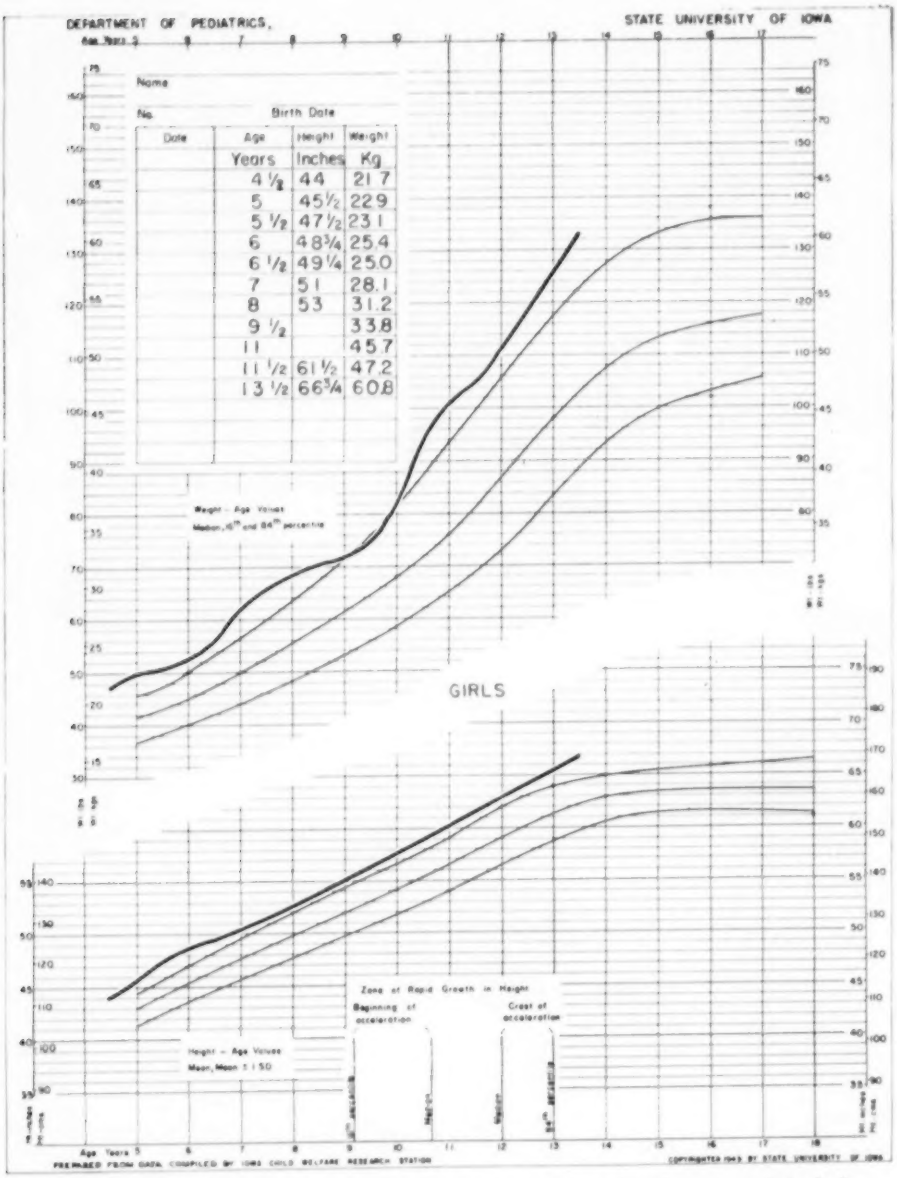


Fig. 4.—The height and weight growth curves of the patient plotted over a period of nine years. In spite of the fact that this child has been clinically ill almost one-third of her life (a period of 5 to 7 days during the neutropenic phase of each 21-day cycle) and has had numerous infections, which on several occasions have been life-threatening, her growth and physical development have always been normal or greater than normal.

body in our patient's serum at various stages of her neutrophil cycle.

Attempts to demonstrate agglutination of normal neutrophils and the patient's own neutrophils were carried out employing the patient's serum in vitro. The three different techniques described by Martensson and Vikbladh,[34] Dausset,[39] and Moeschlin[40]

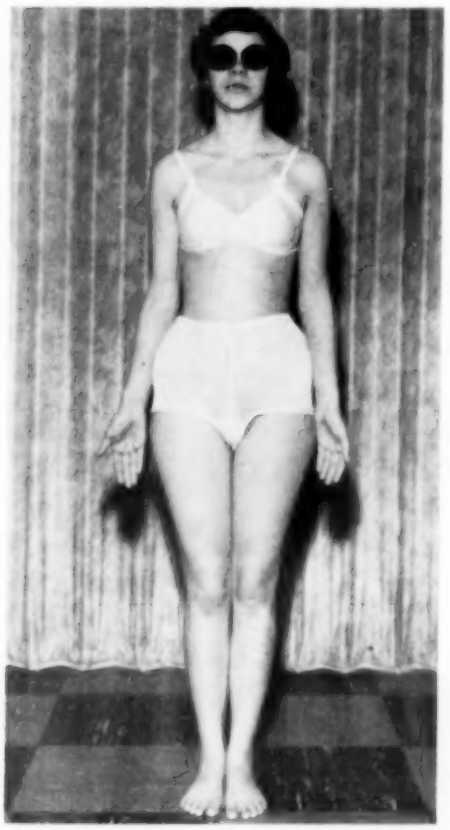


Fig. 5.—The patient at 14 years of age, illustrating normal maturation in spite of her cyclic hematologic disorder.

were used. Serum was tested for agglutinins when the neutrophils were at the lowest levels, just prior to the decline in neutrophil count, during the decline in neutrophil count, and when the neutrophils were at the highest concentration. All efforts to demonstrate an antibody capable of agglutinating the patient's cells or normal white cells have been negative. Further attempts to find incomplete anti-white-cell antibody, by use of the direct Coombs test adapted to the study of leukocytes, were carried out. In these experiments the leukocytes were incubated in the patient's serum and tested for agglutination using anti-human-γ-globulin antiserum. Negative results were again obtained. Unfortunately, in this particular study, technical obstacles often prevented a satisfactory test of the possible existence of antibody, since washing of the cells sufficient to remove nonspecific globulins from the surface resulted in damage to the white cells and spontaneous nonspecific agglutination occurred. However, no evidence of the existence of an incomplete antibody was found in the few successful experiments which were performed.

Attempt to Demonstrate Circulating Factor in Blood and Serum.—Moeschlin [28] has been able to produce leukopenia in normal subjects by injecting serum from patients with agranulocytosis. In his studies, injection of 300 cc. of blood from patients with agranulocytosis produced agranulocytosis in the normal recipient which lasted as long as four hours after the injection.

To exclude the participation of an antibody directed against neutrophils in the development of cyclic neutropenia, three additional experiments were performed. Whole blood (250 cc.) taken from our patient during the neutropenic phase was injected into a compatible recipient with myelogenous leukemia. In another experiment 100 cc. of serum taken from our patient during the neutropenic phase was injected intravenously into a hematologically normal child. In another experiment 150 cc. of plasma taken from our patient at the neutropenic phase of her cycle was injected intravenously into a child with a slight polymorphonuclear leukocytosis. In still another study, transfusion of 400 cc. of whole blood from our patient into a compatible normal recipient was carried out. White blood cell counts and differential counts taken serially during the succeeding 48-hour period revealed no significant fall in the numbers of neutrophilic cells in any instance.

Even though we could not demonstrate agglutination or rapid destruction of normal neutrophils by the patient's serum which might be attributable to a circulating antibody or neutrophilic toxin, the morpho-

logical characteristics of our patient's cells, showing "toxic granulation" as they did (Fig. 2), suggested that a factor toxic to these cells circulating in the peripheral blood should be ruled out.

Attempt to Demonstrate Damage to Neutrophils.—In an effort to achieve a more definitive evaluation of the possibility that the circulating neutrophils might be undergoing damage, recourse was made to methods of evaluating neutrophilic function. The functions we chose to study were motility and phagocytosis.

To study motility, comparisons were made between the rate of migration of the patient's white cells and that of normal white cells. In addition, the effect of the patient's serum on migration of normal white blood cells was investigated. In this study the effect of serum taken from the patient at both high and low points of the neutrophil cycle was compared with that of normal serum.

Leukocyte Migration

Pierce-Martin[9] cells were constructed by sandwiching a piece of Parafilm cut in U-shape between a clean glass slide and a cover-slip. The cells were tightly sealed by applying light pressure and placing in a 150 C oven for 15 minutes.

Silicone-coated glassware was used throughout the experiment. Fresh venous blood containing 2 units of heparin per cubic centimeter was taken from our patient and from a normal control with the same blood type. At 4 C, the bloods were centrifuged at 500 rpm for 30 minutes and the cells and plasma were separated. Various combinations of cells and plasma were made by mixing equal parts of such. Several Pierce-Martin cells were filled with each combination by applying the tip of a capillary pipette to the corner of the open surface of the cell. The cells fill by capillary attraction, much like a counting chamber. The cells were then centrifuged at 1500 rpm for 20 minutes and incubated at 37 C in an upright position. During centrifugation the blood separates into a red cell phase, white cell phase, and plasma. The plasma, kept in a liquid phase by minimal concentration of heparin and by working at low temperature over a short interval, clots soon after the beginning of the incubation at 37 C, so that a fibrin network is formed on which the leukocytes migrate. Measurements of the distance between the boundary of migrating leukocytes and the red cell layer are then made at intervals of one hour on a microscope equipped with a graduated mechanical stage. The measurements are reproducible within limits of ±0.1 mm. Figure 6 illustrates the appearance of a fully charged Pierce-Martin cell following centrifugation.

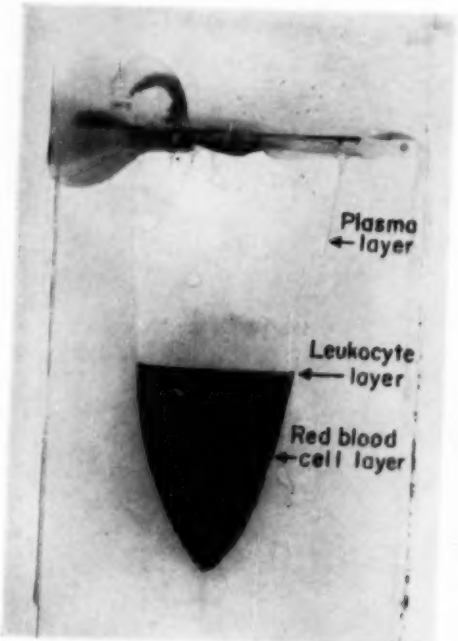


FIG. 6.—Appearance of a fully-charged Pierce-Martin cell after centrifugation. The motility of the white blood cells is evaluated by measuring the distance of their migration out into the plasma layer from the buffy coat under controlled conditions of temperature and postbleeding timing.

The entire experiment was done twice, once when our patient had no neutrophils in her blood and again when normal numbers of neutrophils were present. Photographs were taken three hours after the beginning incubation.

Results. The results, summarized in Tables 2 and 3, show that our patient's plasma does not noticeably affect the migra-

TABLE 2.—*Effect of Cyclic Neutropenia on Migration of Leukocytes*
Neutrophils Absent from Blood

Pierce-Martin Preparation	Distance of Migration of Leukocytes, Mm.		
	1 Hr.	2 Hr.	3 Hr.
Normal cells + normal plasma	0.3	0.7	1.0
Normal cells + "neutropenic" plasma	0.4	0.8	1.0

TABLE 3.—*Effect of Cyclic Neutropenia on Migration of Leukocytes*
Neutrophils Present in Blood

Pierce-Martin Preparation	Distance of Migration of Leukocytes in 2 Hr. Mm.
Normal cells + normal plasma	0.7
Normal cells + patient's plasma	0.6
Patient's cells + normal plasma	0.8
Patient's cells + patient's plasma	0.6

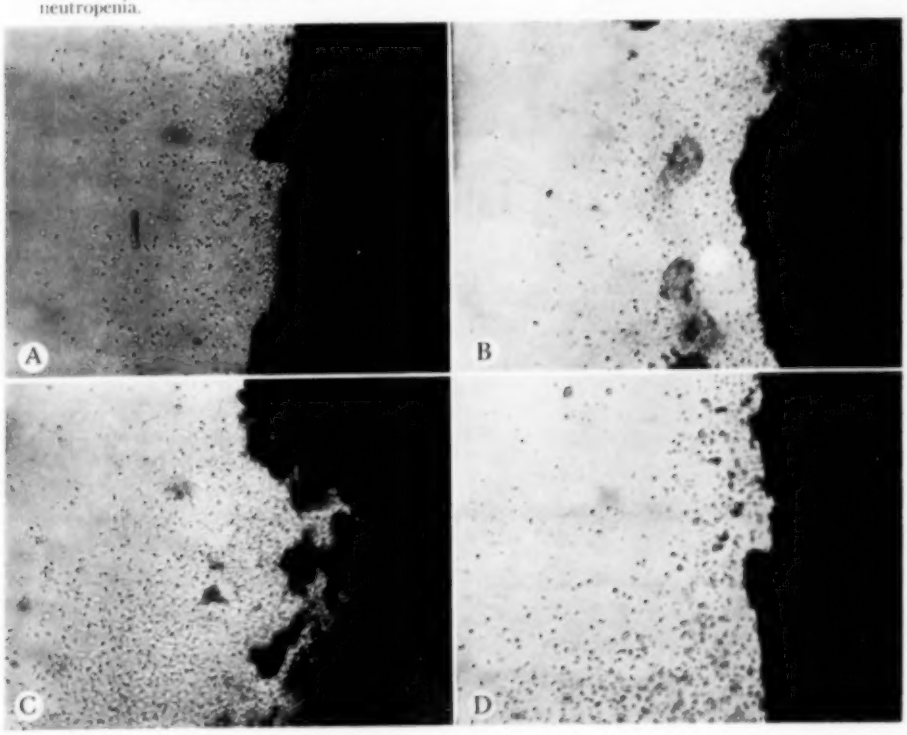
tion of normal cells and, further, that her own cells migrate at a normal rate in her own and in normal plasma. Figure 7 illustrates the appearance of the migrating leukocyte boundary in each of the preparations studied.

Phagocytosis

In a comparable study, the phagocytic capacity of the patient's cells and the effect of the patient's serum on phagocytic capacity of normal cells was evaluated by use of Wood's method of surface phagocytosis.[42] Again, studies were made with serum taken at various stages of the neutrophilic cycle and comparisons with the effect of normal serum were made.

Method. Type I Pneumococcus (SV_1) was grown in brain-heart infusion (BHI) broth. An 18-hour culture was used for this experiment. Neither the patient's serum nor the control serum used gave a positive quellung reaction with these organisms. Consequently, it was concluded that no specific antibodies against Type I pneumococci were present in either serum sample at this

Fig. 7.—Leukocyte migration after three hours of incubation in Pierce-Martin cells; ×100. *A*, the patient's cells taken during the neutrophilic phase of the cycle and combined with normal serum. *B*, the patient's cells combined with her own serum which had been taken during the neutropenic phase of her cycle. *C*, normal cells combined with normal serum. *D*, Normal cells combined with the patient's serum taken during the neutropenic phase of her cycle. In this study no evidence of an abnormality of the patient's cells nor of a circulating substance capable of stimulating or interfering with motility of normal neutrophils or neutrophils from the patient was found during the period of maximal neutropenia.



time. Siliconized glassware was used throughout the experiment.

The leukocytes were obtained by adding human fibrinogen to heparinized blood to increase the rate of sedimentation of the red blood cells. After one hour of sedimentation the plasma layer was drawn off. This layer is rich in leukocytes and contains few red cells. These cells were then separated from the plasma by gentle centrifugation for 15 minutes at 500 rpm. The packed white blood cells were then used for the study of phagocytic capacity.

Fifteen cubic centimeters of an 18-hour BHI broth culture of Type I pneumococci was divided into three equal parts. The tubes were centrifuged at 1500 rpm for 30 minutes, and the sediment was resuspended in 1 cc. of the serum to be studied. This mixture was then added to approximately 0.1 cc. of the packed leukocytes to be studied. The cells were resuspended in the serum-pneumococcal mixture and incubated for 15 minutes at 37 C. Then this mixture was spun at 1000 rpm for 15 minutes and the supernatant was discarded. The sediment was incubated an additional 30 minutes at 37 C, and smears were prepared and stained with Wright's stain.

In this manner, the patient's cells taken when she had neutrophils were incubated in the patient's serum taken when she had no neutrophils and compared with her cells incubated in normal serum. The average number of pneumococci in each cell was determined by counting the number of organisms in 50 successive neutrophils.

Results

Phagocytosis by Patient's Cells

Preparation	Phagocytosis, Pneumococci/Neutrophil
Patient's serum (neutropenic phase) + patient's cells (neutrophilic phase)	45.9
Normal serum + patient's cells (neutrophilic phase)	37.7

Method. Next, normal cells were incubated with the patient's serum from both high and low points of her cycle and were compared with normal cells incubated in normal serum. The method was the same except that the normal cells were obtained by spinning the blood at 1500 rpm for 30 minutes and using the buffy coat. The organisms in 50

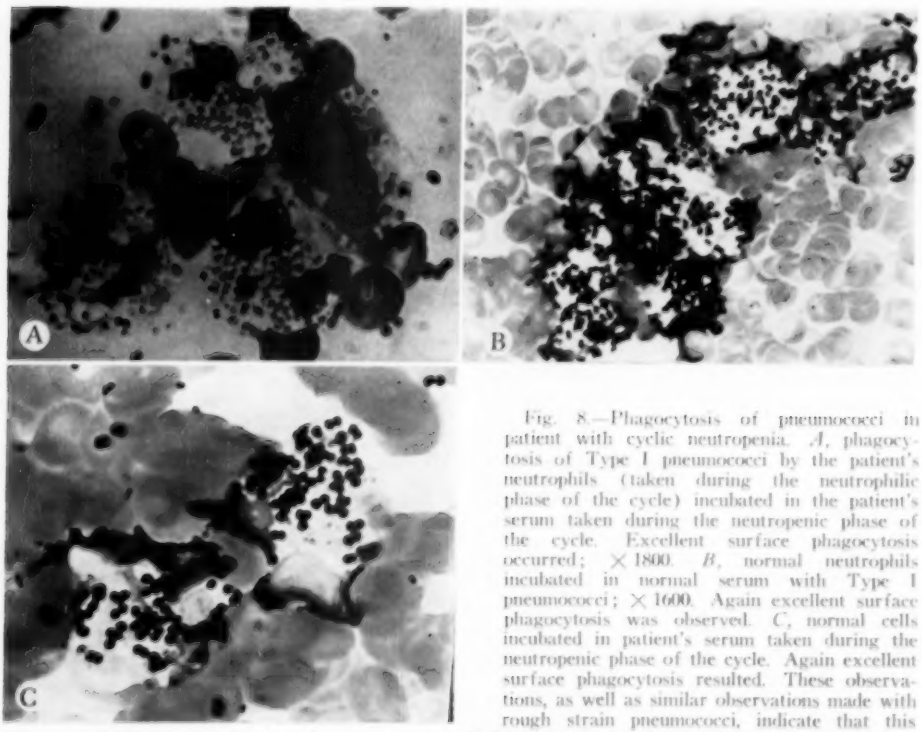


Fig. 8.—Phagocytosis of pneumococci in patient with cyclic neutropenia. *A*, phagocytosis of Type I pneumococci by the patient's neutrophils (taken during the neutrophilic phase of the cycle) incubated in the patient's serum taken during the neutropenic phase of the cycle. Excellent surface phagocytosis occurred; ×1800. *B*, normal neutrophils incubated in normal serum with Type I pneumococci; ×1600. Again excellent surface phagocytosis was observed. *C*, normal cells incubated in patient's serum taken during the neutropenic phase of the cycle. Again excellent surface phagocytosis resulted. These observations, as well as similar observations made with rough strain pneumococci, indicate that this patient's neutrophils are capable of normal phagocytic function when present and that her serum during the neutropenic phase of the disease does not contain a substance toxic to neutrophils that might be revealed by alteration in the phagocytic function of these cells.

neutrophils were counted to obtain an average number of pneumococci per neutrophil.

Results

Phagocytosis by Normal Cells	Pneumococci/Neutrophil
Normal cells + patient's serum (neutropenic phase)	46.7
Normal cells + patient's serum (neutrophilic phase)	47.4
Normal cells + norcal serum	51.2
Normal cells + normal serum	51.2

Figure 8 illustrates the phagocytosis observed with patient's cells in patient's serum (neutropenic phase), normal cells in patient's serum (neutropenic phase), and normal cells in normal serum.

The error for the method is in the order of ±5 pneumococci per cell. Similar studies were done using the D39R rough strain of pneumococci, which revealed entirely similar results. These results demonstrate normal phagocytic activity for the patient's cells and show that the patient's serum does not alter the phagocytic activity of normal cells.

Bone Marrow Studies.—Prior to initiation of the present study, several bone marrow studies had been performed on the patient. They revealed plasmacytosis and eosinophilia and other nonspecific changes thought to be compatible with a chronic infectious process. However, no light was shed on the problem of how changes in neutrophil count in the peripheral blood were reflected in the bone marrow alterations.

As part of this investigation, numerous marrow biopsies have been performed. For example, serial bone marrow studies (four marrow samples over an 18-day period) were done during one complete cycle in hopes that information regarding the basic disease process could be obtained. Both sternum and iliac crest punctures were used. Five hundred cell differential counts were made on the direct smears. Relative cellularity was evaluated by study of myeloid-erythroid layer of the oxalated specimens spun in a hematocrit tube at 3000 rpm for 30 minutes. The differential counts on the marrow samples are summarized in Table 4.

Relating the findings in these marrow samples to one another in a meaningful manner in face of the shifting cellularity was a difficult problem. Because the hemoglobin and red cell count remained constant during the patient's cycles we assumed that the absolute number of normoblasts would remain constant. With the normoblasts used as a reference point, the ratio of the percentage of each cell type in the neutrophil series to the percentage of normoblasts in that particular marrow sample was calculated and a graph of these ratios was constructed.

TABLE 4.—*Differential Cell Counts on Serial Bone Marrow Samples from a Patient with Cyclic Neutropenia*

	Cells, %			
	7/23/54	7/27/54	8/4/54	8/9/54
Myeloid-erythroid layer	2	4	4	No measurement
Myeloblast	1.6	3.0	1.6	2.2
Peomyelocyte	7.0	14.4	9.0	9.0
Neutrophilic myelocyte	1.8	2.6	8.0	0.0
Neutrophilic metamyelocyte	1.2	0.6	8.0	0.0
Mature neutrophil	0.0	0.0	4.8	0.0
Eosinophilic myelocyte	5.0	10.2	5.6	7.2
Eosinophilic metamyelocyte	3.8	5.2	4.4	4.6
Mature eosinophil	4.2	7.6	4.0	2.4
Basophilic myelocyte	0.6	0.8	0.8	0.4
Basophilic metamyelocyte	0.2	0.2	0.4	0.2
Mature basophil	0.2	0.8	0.2	0.6
Lymphocyte	22.6	18.4	13.8	23.8
Megakaryocyte	0.8	0.6	0.8	0.0
Reticulum cell	5.6	6.0	2.8	9.8
Plasma cell	0.8	2.2	2.0	1.8
Pronormoblast	2.4	4.0	5.6	2.0
Normoblast	33.8	14.8	24.8	29.8
Monocyte	9.2	8.6	3.4	6.8

On the basis of the hypothesis that the cyclic disappearance of neutrophils from the circulation in this patient is due to intermittent failure of formation of neutrophils, one might postulate that initially there would occur an arrest at a primitive stage of the neutrophilic sequence, which would be followed by successive disappearance from the bone marrow of the subsequent maturational stages. With reconstitution of bone marrow function following such arrest one might anticipate that evidence of neutrophil maturation would again be observed, with reappearance first of the immature forms and successive replacement of the more mature forms until fully mature neutrophils are again being produced. The serial studies of the bone marrow during the

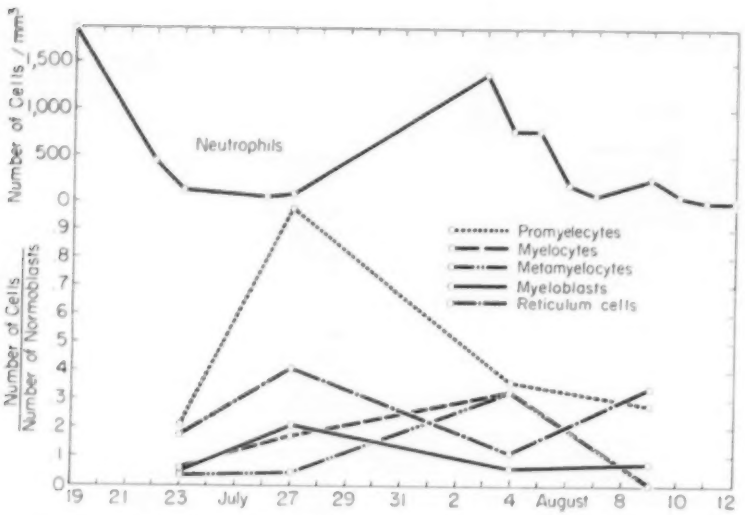


Fig. 9.—The relationship of circulating neutrophils to changes in concentration of neutrophilic precursors in bone marrow. Note that at the time of maximum neutropenia all of the precursors of neutrophils are deficient in number. Indeed, when the neutrophils are absent from the circulation, myelocytes and metamyelocytes are almost completely absent from the marrow. Late in the neutropenic phase of the cycle just prior to rise in the numbers of circulating neutrophils there appears to be an increase in numbers of myeloblasts, reticulum cells, and myelocytes and a marked increase in numbers of promyelocytes. Just prior to the cyclic decline in numbers of neutrophils the reticulum cells, myeloblasts, and promyelocytes decrease in concentration, a change which is followed by a disappearance of bone marrow myelocytes, metamyelocytes, and circulating neutrophils. These observations indicate that cyclic changes occurring in the bone marrow anticipate the characteristic changes in the peripheral blood.

cycles of neutropenia provide strong evidence that this is exactly what takes place.

As revealed in Figure 9, during the period preceding increase in the numbers of neutrophils in the circulating blood, peak levels of reticulum cells are reached prior to the increase in numbers of myeloblasts and promyelocytes, and increased numbers of these cells are followed by the appearance of increasing numbers of the myelocytes and metamyelocytes and neutrophils in the bone marrow. At the time when the polymorphonuclear leukocyte count is declining in the peripheral blood, virtually no myelocytes nor metamyelocytes are to be found in the marrow, the promyelocytes are at their lowest level, and the primitive reticulum cells reach their highest concentration.

These observations would be compatible with the hypothesis that basically the cyclic disease process is reflected in the bone marrow as an intermittent failure of heteroplastic maturation of the neutrophil series and that the cycles observed in the peripheral blood are secondary to the intermittent maturational failure and subsequent reconstitution of cellular production observed in the bone marrow.

Although the data from the four bone marrow specimens taken during a typical cycle are insufficient to establish this hypothesis firmly, the results of study of 14 additional marrow samples taken at various phases of the cycle are compatible with this hypothesis and, indeed, are best explained by it.

All of the marrow samples taken at the beginning phase of peripheral blood neutropenia have shown absence of myelocytes, metamyelocytes, and mature neutrophils. In addition, the promyelocytes are at the lowest levels at this time. Marrow samples taken just prior to the appearance of neu-

trophils in the peripheral blood contain numerous myelocytes and metamyelocytes, and at this time the promyelocytes are greatly increased. Finally, the marrow samples taken during the decline of the neutrophil count lack neutrophils, myelocytes, and metamyelocytes, and promyelocytes are at relatively low levels.

One observation from the above studies which appears to us to be of great interest, but served to confuse this picture, was the finding that the bone marrow of this patient constantly showed excessive numbers of promyelocytes. This observation might be interpreted to indicate a "maturational block" at the promyelocyte stage. If this were the case, then the fluctuation in numbers of reticulum cells and myeloblasts would be left without explanation. Further, if arrest of maturation of neutrophils at the promyelocyte stage were invoked as a morphological mechanism to explain the cyclic neutropenia, it would appear that the peaks and depths of the promyelocyte cycle would be out of phase with the peaks and depths of the neutrophilic cycle. It is possible that a combination of intermittent general failure of development of neutrophils combined with a persistent maturational deficiency at the promyelocyte level even during the neutrophilic phase of the cycle would explain all of the findings in this study. Thus, during the entire cycle the relative numbers of promyelocytes in the bone marrow would be too large; during the period of decline in the numbers of circulating neutrophils the myelocytes and metamyelocytes would be virtually absent from the marrow and the promyelocytes would be fewer than at any other time. With return of neutrophilic production prior to the appearance of the neutrophils in the circulation, the promyelocytes would increase to inordinate levels, followed by successive appearance of myelocytes, metamyelocytes, and neutrophils in the marrow and then neutrophils in the peripheral blood. This, in fact, is what has been observed (Fig. 9).

Regardless of the responsible mechanism, the increase in promyelocytes during the neutropenic phase of the cycle is real and constant. As a matter of fact, its constancy has provided a basis for evaluating the effect of attempted treatment of the disease process. For example, when the patient was treated with corticotropin (Fig. 13) and a rise in promyelocyte levels occurred during the neutropenic phase of the peripheral blood cycle, this was taken as evidence that corticotropin did not alter the underlying disease process. Indeed, in spite of corticotropin therapy the fluctuation in numbers of circulating leukocytes occurred with the same sequence and time relationships as when no treatment was given. Again, during an unsuccessful attempt to alter the disease by the parenteral injection of leukocytes during the neutropenic phase the numbers of promyelocytes in the bone marrow increased to a peak level just prior to the return of neutrophils to the circulation. The details of the latter two experiments will be discussed below.

Hormone Studies.—Since the regular cyclic nature of the disease as well as the necrotizing process involving the mucous membranes suggested a possible relation of the disease process to the menstrual cycle, we postulated that the cyclic variation of the neutrophils might result from a hormonal disturbance. In an initial effort to test this theory we recorded the patient's morning basal rectal temperature, periods of menstruation, and neutrophil counts over a four-month period. As can be seen in Figure 10, the menstrual periods bear no consistent relationship to the neutrophil cycle. Indeed, during the four-month period of this study, menstruation began at almost all the possible phases of the neutrophilic fluctuation. In the initial cycle menstruation began when neutrophils had just disappeared from the blood; in the second cycle menstruation began when the neutrophil count was rising rapidly; in the third cycle menstruation began when the neutrophils were just beginning to rise, and

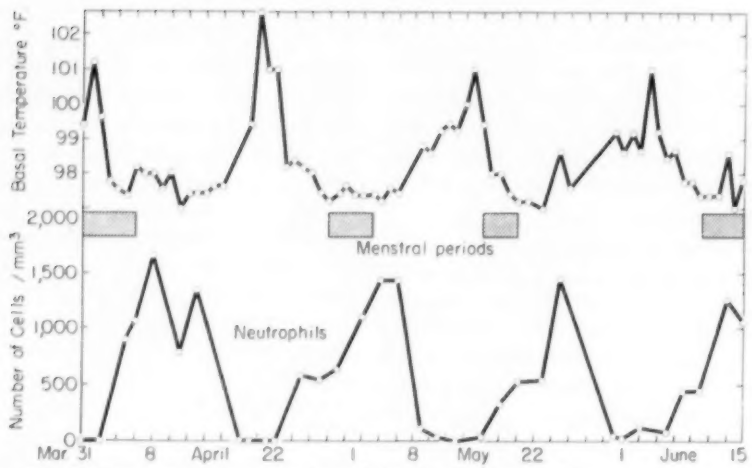


Fig. 10.—Relation of menstrual cycle and basal temperature changes to the neutrophil cycle in a patient with untreated cyclic neutropenia. The regular occurrence of fever associated with the cyclic fluctuation of neutrophils negates the value of this measurement in establishing time of ovulation. During this period of study, illustrated by the graph, it may be observed that onset of menstruation bears no relationship to the cyclic neutropenia in this patient.

in the fourth cycle, when the neutrophilic cycle had reached its peak. The temperature curve shows that spikes of fever regularly accompany the neutropenic episodes and consequently render the basal temperature useless as an indicator of ovulation time. Further support for the dissociation of the cyclic neutropenia from menstruation has been obtained from the history and further clinical observation. The patient has been menstruating for three years, but, despite the fact that she has been oriented to the possibility of a relationship between menstruation and her cyclic disease, it is her firm conviction that no clinical relationship exists. Additional clinical observations during the year the patient was studied in the hospital indicate that menstruation has begun at every possible locus on the curve describing the neutrophilic cycle.

Although these data demonstrate clearly that the cyclic neutropenia is not related to menstruation, its periodicity led us to investigate further the possibility that endocrine function might be involved in control of neutrophil maturation and that an endocrine disturbance is basic to this disease. Since the process was cyclic we felt that only a disturbance in a central control mechanism was likely.

We reasoned further that serial determinations of adrenal-pituitary and adrenal-gonadal activity during the various phases of the neutrophil cycle might reveal abnormalities if the cycle was under hormonal control. To evaluate these activities, determinations of urinary follicle-stimulating hormone (FSH) and 17-ketosteroid excretion, blood corticotropin concentration, and circulating 17-hydroxycorticosteroid (17-OHS) and eosinophil response to corticotropin were made. All of the determinations were done during both the neutrophilic and neutropenic phases of the patient's cycle. The results of these determinations are summarized in Table 5. The urinary FSH excretion was less than 6 m. u. (mouse units) per 24 hours during the neutropenic phase and less than 12 m. u. per 24 hours during the neutrophilic phase of the cycle. These are not significant differences, and both values are within the normal limits for the patient's age group. The urinary 17-ketosteroid excretion was 6.2 mg. per 24 hours during the neutropenic phase and 4.0 mg. per 24 hours during the neutrophilic

TABLE 5.—*Hormonal Studies in Cyclic Neutropenia*

	Urine FSH Titer,* M. U./24 Hr.	Urine 17-Ketosteroids,† Mg./24 Hr.	Corticotropin Eosinophil Response Test, Cells/Cu. Mm.		Corticotropin 17-OHS Response Test ‡			Corticotropin Activity §
			Control	4 Hr.	Control	1 Hr.	2 Hr.	
Neutrophils at low point of cycle	6	6.2	556	181	8.3	..	26.2	None
Neutrophils at high point of cycle	12	4.0	421	272	10.5	30.1	33.4	None

* FSH determination done by the method of McCullogh and Klinefelter.
† 17-Ketosteroid determination done by the method of Drekter and Klendshoj.
‡ 17-Hydroxycorticosteroids measured by method of Nelson and Samuels.
§ Adrenal ascorbic acid depletion method of Sayers et al.

phase of the cycle. Both of these values fall within the normal range, and the difference in the values can be accounted for by the variability of the method. At both extremes of the cycle the adrenal response to the intramuscular injection of 25 mg. of corticotropin was normal as measured by the eosinophil depletion and response of 17-hydroxycorticosteroid serum concentration. No corticotropin activity was measurable during either the neutropenic or the neutrophilic phase of the patient's cycle. This, too, is a normal finding.

These studies make it seem unlikely that the pituitary-gonadal or pituitary-adrenal systems are involved in the basic disturbance responsible for our patient's disease. Although these observations fail to implicate pituitary function in the cyclic neutropenia, they do not eliminate the possibility that a central control mechanism involving pituitary function at some other level might be a controlling factor in this disease. Recent studies [43] suggest that the pituitary gland does exert control of factors involved in production of leukocytes and red blood cells. However, no conservative way of further testing this hypothesis could be devised with techniques presently available, and from our observations we could not implicate a hypothalamic-pituitary mechanism in this disease.

Other Studies.—Electrophoretic studies of our patient's serum regularly revealed the γ-globulin level to be higher than normal. This finding prompted the extensive search for leukoagglutinins mentioned above, which was unsuccessful. To further explore the possibility that the hypergammaglobulinemia was causally related to the cyclic neutropenia, quantitative determinations of the γ-globulin were done on the patients serum.

The γ-globulin concentration was estimated from the total serum protein determined by a standard biuret technique and the percentage of γ-globulin as revealed by paper electrophoresis. The electrophoretic patterns were made on a Spinco electrophoresis apparatus, and their analysis was done automatically with use of a Spinco Analytrol and automatic integrator. With this method, results reproducible within ±0.05 gm. of γ-globulin were obtained.

These determinations were done during the neutropenic and neutrophilic phases of a normal cycle, and a series of three determinations were done during one cycle while the patient was receiving steroid therapy. The results show that, although regularly elevated, the γ-globulin concentration did not vary significantly from one phase of the cycle to another. The elevated γ-globulin concentration was associated with an increased number of plasmacytes in the bone marrow. During a period of steroid therapy, however, a progressive decrease in γ-globulin levels occurred, which was associated with a decrease in the number of plasma cells in the marrow. In spite of this change, the neutrophilic cycle continued in the usual manner. The results of this study are summarized in Figure 11. It is our interpretation that these findings, although demonstrating a γ-globulin dysmetabolism in this disease, clearly eliminate γ-globulin and plasma cells as basic participants in the development of cyclic neutropenia in this patient.

A search for variations in other serum components led us to do serial determinations of the erythrocyte sedimentation rate,

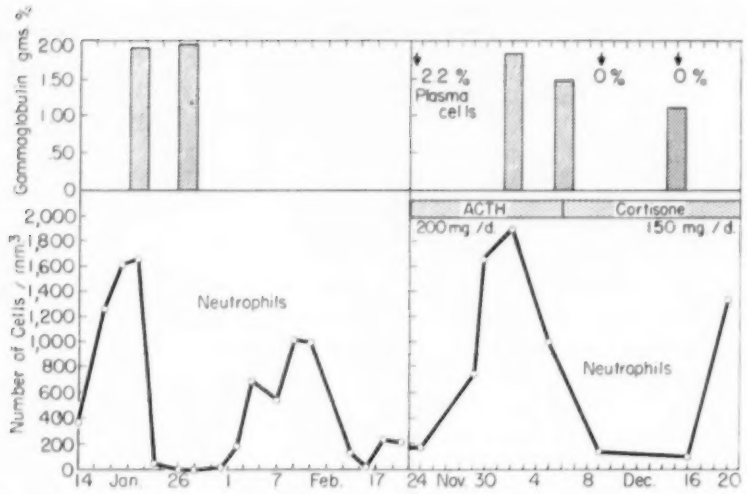


Fig. 11.—Relationship of bone marrow plasma cells, γ-globulin concentration, and cyclic neutropenia. On the left-hand graph in this Figure it is shown that this patient had an increased γ-globulin concentration which did not fluctuate during the cyclic variations in the neutrophil count. On the right-hand graph it is shown that large-dose steroid treatment resulted in a marked decrease in plasma-cell concentration and reduction in γ-globulin concentration but had no effect on the cyclic neutropenia. This observation would tend to dissociate the cyclic neutropenia from the plasmacytosis and γ-globulin elevation observed in this patient.

C-reactive protein, mucoprotein, fibrinogen, and heparin-precipitable fraction (HPF of Thomas, Smith, and Von Korff [44]) in the patient's serum during one neutrophil cycle. As shown in Figure 12, the patient has a moderate persistent elevation of the sedimentation rate, probably on the basis of the elevated serum γ-globulin level. Her mucoprotein, fibrinogen, and HPF levels remained within normal levels and did not

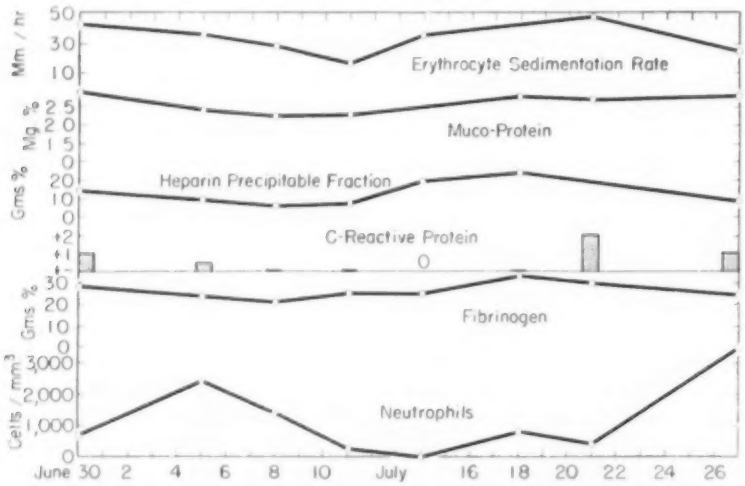


Fig. 12.—Serial determination of acute-phase reactants in a patient with cyclic neutropenia. Persistent elevations in the sedimentation rate and appearance of C-reactive protein, heparin-precipitable protein, and fibrinogen concentration showed no consistent relationship to the fluctuating concentration of neutrophils in the peripheral blood.

show significant variation. The C-protein was normal until late in the neutropenic phase of the cycle. The C-protein elevation that occurred at this time is not a constant finding and might well be attributed to intercurrent infection. The absence of elevation in the levels of these acute-phase reactants throughout the period studied is evidence that the patient is not suffering from a chronic infectious process nor unusual form of inflammatory disease producing secondary depression in polymorphonuclear leukocyte concentration.

Pillemer [45,46] has recently described a nonspecific bactericidal factor in the serum of man and animals which he feels is of real significance as a natural defense mechanism against infection with various microorganisms and intoxication by bacterial products. Ross et al.[47] have shown that the properdin concentration of serum decreases to extremely low levels following total body irradiation. This decline occurs when the neutrophil count is low as a result of interference with heteroplastic development of neutrophils from the hematopoietic reticulum. Animals both neutropenic and properdin-deficient were found to be extremely susceptible to infection, and the authors proposed that death following total body irradiation might often be a function of properdin deficiency. To test this hypothesis they injected purified properdin into the irradiated animals and demonstrated enhanced resistance to infection and markedly decreased lethal effect of Gram-negative micro-organisms as a consequence of this treatment.

Since the fall of properdin concentration in the irradiated animals might be attributable to deficiency of leukocytes, we postulated that this biologically active substance might be produced by neutrophils or its liberation into the blood might be associated with the maturation or function of these cellular elements. Consequently, we decided to study the total properdin concentration in this patient with cyclic neutropenia to determine whether properdin concentration was related in any way to the activities of neutrophils or associated with the formation of polymorphonuclear leukocytes. Another reason for our desire to study the properdin concentration during the neutropenic phase of the cycle was our impression that in this patient and in other reported cases of cyclic neutropenia the susceptibility to infection during the neutropenic phase did not appear to be as great as in patients with idiopathic agranulocytosis, agranulocytosis associated with drug reaction, or agranulocytosis following x-ray therapy.

The total properdin concentration was measured during the period of extreme neutropenia and again when the neutrophils were present at nearly normal levels. The results, summarized in Table 6, reveal that both determinations of properdin were within the normal range and that no variation in concentration of properdin occurred to reflect the fluctuations in the numbers of circulating leukocytes.

Experimental Studies on the Functions of the Neutrophil

Since the patient has a disease in which the neutrophil count fluctuates from extremely low levels to levels approaching normal, she represents what might be considered an ideal system in which to study the function of neutrophils and to evaluate the role of these cells in the body economy. For example, the response of the patient to a stimulus being investigated may be studied at a time when the patient possesses no neutrophils in the blood or blood-forming tissues and again when neutrophils are abundant in the bone marrow and peripheral blood. In this way, critical evaluation of neutrophil function may be made with the patient serving as her own control. Among

TABLE 6.—*Relation of Serum Properdin Concentration to the Neutrophil Count in a Patient with Cyclic Neutropenia*

Neutrophil Count, Cells/Cu. Mm.	Properdin Concentration, Units/Ml.
1600	5
0	6

the neutrophilic functions which have been studied by making use of this "experiment of nature" are (1) role of the neutrophil in antibody production, (2) role of neutrophil in the development of acute-phase serological reactions, (3) role of the neutrophil in development of local reactions to Gram-negative bacterial endotoxins, (4) role of the neutrophil in local reactions of bacterial-type hypersensitivity, (5) role of the neutrophil in the development of systemic reactions to Gram-negative bacterial endotoxins, (6) role of the neutrophil in the development of refractoriness to Gram-negative bacterial endotoxins, and (7) role of the neutrophil in the inflammatory response.

Role of the Neutrophil in Antibody Production.—Although current literature clearly establishes the production of antibody at an extrahepatic site [48,49] and implicates cells of the reticuloendothelial system (reticuloendothelial phagocytes, lymphocytes, and plasma cells) [50-52] as the basis for this physiological function, no clear evidence has been presented in man or experimental animals excluding the polymorphonuclear leukocyte as a participant in this important activity. Since inflammation regularly accompanies local exposure to antigenic material and since the polymorphonuclear leukocyte plays an essential role in the inflammatory response to all antigens thus far studied, it is conceivable that neutrophils might play a significant role in the formation of antibody. Direct and indirect evidence linking plasma cells [53-56] and possibly lymphocytes [57,58] with the production and liberation of antibody is overwhelming. Although no evidence has been presented linking the neutrophil with this function, it was postulated that should the neutrophil play an essential role in antibody formation such function would be associated with preparation of antigen for utilization by the cells of the lymphoid system. To test this hypothesis, the patient was subjected to antigenic stimulation with typhoid-paratyphoid vaccine during a period of persistent neutropenia and the immune response was measured by quantitative evaluation of the formation of agglutinating antibodies directed against the H, O, and B antigens of the organisms injected.

Summarized in Table 7 are data comparing the immune response observed in the patient during a period of agranulocytosis following injections of typhoid paratyphoid vaccine with the immune response of three normal children to comparable antigenic stimulation. It will be seen in the Table that the immunological response observed

TABLE 7.—*Formation of Antibody During Period of Complete Neutropenia in Child with Cyclic Neutropenia Compared with That of Normal Children*

	Antigenic Stimulation	Antibody Titer					
		H Antigen		O Antigen		B Antigen	
		Initial Titer	2 Wk. After Last Injection	Initial Titer	2 Wk. After Last Injection	Initial Titer	2 Wk. After Last Injection
The patient, 16-yr.-old girl, cyclic neutropenia, neutropenic phase *	50,000,000 S. typhosa, 250,000,000 paratyphosa organisms subcutaneously, 650,000,000 T A B organisms i. v. over 2 wk. period	0	1:5,120	0	1:1,280	0	1:2,560
12-yr.-old normal boy, plastic repair	500,000,000 S. typhosa, 250,000,000 paratyphosa organisms subcutaneously, 600,000,000 T A B organisms i. v. over a 2 wk. period	0	1:10,240	0	1:1,280	0	1:2,560
10-yr.-old normal boy ambulatory phase of healing fracture	50,000,000 S. typhosa, 250,000,000 paratyphosa organisms subcutaneously, 650,000,000 T A B organisms i. v. over a 2 wk. period	0	1:2,560	0	1:640	0	1:2,560
6-yr.-old normal child		0	1:10,240	0	1:2,560	0	1:5,120
5-yr.-old girl, agranulocytosis †		0	1:5,120	0	1:5,120	0	1:2,560

* Neutrophils were absent or virtually absent from the circulation and bone marrow during the entire period of study.
† Persistent agranulocytosis.

in this child did not differ significantly from that of normal children given comparable stimulation with these antigens. Also included in the Table are data describing the immunological response in a patient suffering from severe persistent agranulocytosis and aplastic anemia. Just as was the case in the patient with cyclic neutropenia, virtual absence of neutrophils from the circulation and bone marrow did not interfere with the formation of circulating agglutinins. Subsequently, normal response to diphtheria toxoid, mumps virus, and poliovirus antigens was obtained. In this way it was demonstrated that the complete absence of neutrophils from the blood and blood-forming tissues does not interfere with the production of antitoxic and agglutinating antibody against protein antigen, lipopolysaccharide antigens, or the complement-fixing and virus-neutralizing antibodies against inactivated virus agents.

Role of the Neutrophil in Development of Acute-Phase Serological Reactions.—A number of changes occur in the blood during the acute phase of many diseases. Certain of the changes which occur are characteristically present during acute illness, and a return to normal is associated with clinical recovery from the illness in question. These serological changes have been referred to as acute-phase reactions.[59,60] Included with this group of changes in the blood associated with acute disease are changes bringing about (1) an elevation of the erythrocyte sedimentation rate; (2) increased concentration of fibrinogen in the plasma; (3) increased concentrations of α-globulins, polymeric carbohydrate protein complexes, hexosamines, and mucoproteins in the serum, and (4) increased amounts of a heparin-precipitable component in the plasma, described by Thomas, Smith, and Von Korff.[44] In addition, the acute phase of many diseases results in the appearance in the serum of a protein not normally present in the blood—namely, the C-reactive protein.[61-63]

Because these changes of the blood serum and plasma often so closely parallel the presence and degree of acute disease activity, each of these substances has been used at one time or another as a clinical indicator of the presence and degree of activity of disease.[60,61,65] These reactions have been particularly helpful in evaluating and following disease activity in rheumatic fever and other so-called collagen diseases.

For many years prior to discovery of any of the "acute phase phenomena" the leukocyte count and, particularly, the absolute polymorphonuclear leukocyte count served as an "acute phase reaction." Indeed, in many diseases it has been observed that there is a positive correlation between the number of circulating neutrophils and the presence of the other so-called acute-phase reactions. Consequently, it seemed of value to determine whether any of the acute-phase reactions currently being studied in our laboratory were dependent on the availability of polymorphonuclear leukocytes. In an initial effort to test the hypothesis that the acute phase reactions might be dependent upon the presence of polymorphonuclear leukocytes in the blood or blood-forming tissues, study was made of the appearance in the serum of C-reactive protein, increase in serum concentration of mucoprotein, and increase in the sedimentation rate following administration of Gram-negative bacterial endotoxin to our patient with cyclic neutropenia during the neutropenic phase of her disease.

Summarized in Table 8 are the results of this study. As may be observed in the Table, injection of endotoxin by vein in the form of typhoid-paratyphoid vaccine in this patient lacking neutrophils in her peripheral blood and bone marrow resulted in a vigorous production or liberation of C-reactive protein and mucoprotein and a significant increase in the erythrocyte sedimentation rate. Similarly, in another patient, suffering from profound persistent agranulocytosis and aplastic anemia of an idiopathic type, the acute-phase reactions

TABLE 8.—*Response of Acute-Phase Reactants to Injection of Endotoxin in Patients with Neutropenia*

Patient *	C-Reactive Protein		Mucoprotein, Tyrosine, Mg./100 Cc.		ESR, Mm. in 60 Min.	
	Before Injection	After Injection	Before Injection	After Injection	Before Injection	After Injection
The patient, 15-yr.-old girl, cyclic neutropenia						
Neutropenic phase	Neg.	6+	5.05	9.23	51	81
Neutrophilic phase	Neg.	4+	4.20	7.80	42	58
5-yr.-old girl, agranulocytosis and aplastic anemia	1+	6+	5.25	10.1	31	54
12-yr.-old boy, normal, plastic surgery	0	4+	2.65	5.9	7	33

* All patients were injected intravenously with 20,000,000 TAB organisms.

occurred as in the normal person. These observations serve to dissociate the acute-phase serological reactions from "acute phase" neutrophilia and indicate clearly that polymorphonuclear leukocytes are not essential to the production or liberation of C-reactive protein, mucoprotein, or the acute-phase reactant responsible for elevation of the erythrocyte sedimentation rate during acute disease.

Role of the Neutrophil in Development of Local Reactions to Gram-Negative Bacterial Endotoxins.—The intradermal injection of Gram-negative bacterial endotoxins results in normal persons in the development of a local lesion featured by erythema and induration. This reaction begins between 6 and 8 hours after injection, reaches a maximum between 24 and 48 hours, and then subsides during the subsequent 48 hours. Microscopic examination of the lesion produced in this way reveals an intense inflammatory response featured by early invasion of the skin by large numbers of polymorphonuclear leukocytes followed by monocytes and lymphocytes. Twenty-four hours after injection, macrophages are abundant in the exudate. The inflammatory response produced by the injection of Gram-negative bacterial endotoxins has, in man and experimental animals, striking similarities to the tuberculin reaction.[69] In rabbits the inflammatory reaction produced by intradermal injection of Gram-negative bacterial endotoxin is associated with "preparation" of the skin for the dermal Shwartzman reaction. The state of "preparation" for the Shwartzman reaction has been shown to be a function of altered metabolism of the skin,[71] which appears to be dependent on infiltration with polymorphonuclear leukocytes.[72]

In light of these observations, it was considered to be of importance to study the skin reactions to Gram-negative endotoxin in our patient with cyclic neutropenia during a period when neutrophils were absent from the blood and bone marrow and again when normal numbers of neutrophils were available. In these experiments the reaction to two different endotoxins and to streptokinase-streptodornase were compared at the high point and low point of the neutrophil cycle. Streptokinase-streptodornase was

TABLE 9.—*Role of the Neutrophil in Local Reactions to Gram-Negative Bacterial Endotoxin and Bacterial-Type Hypersensitivity*

	Polymorphonuclear Leukocytes 0/Cu. Mm.		Polymorphonuclear Leukocytes 42%		Polymorphonuclear Leukocytes 9%		Polymorphonuclear Leukocytes 8%	
	Erythema	Induration	Erythema	Induration	Erythema	Induration	Erythema	Induration
TAB, 0.2 cc. i. d.*	+++	+++	+++	+++	++++	+++	++++	++++
Piromen, 5 μg. i. d.†	None given	None given	+++	++	++	++	None given	None given
SK-SD, 0.1 cc. containing 10 u. SK & 2.5 u. SD i. d.‡	++	++	+++	+++	None given	None given	None given	None given

* Typhoid-paratyphoid vaccine (Wyeth) containing 1,000,000,000 typhoid bacilli, 250,000,000 paratyphoid A bacilli, and 250,000,000 typhoid B organisms.
† Polysaccharide toxin supplied by Baxter & Co.
‡ Streptokinase-streptodornase (Lederle).

Fig. 13.—Delayed skin reactions in a patient with cyclic neutropenia. This patient showed positive skin reactions characterized by erythema and induration to streptokinase streptodornase (*A*), Piromen (*B*), and typhoid-paratyphoid antigens (*C*) in both the neutropenic (above) and neutrophilic (below) phases of her cycle.

chosen as a prototype of a ubiquitous form of the delayed or bacterial type of allergy. The results are summarized in Table 9, where it will be seen that in gross appearance the inflammatory reaction produced by all three agents was no different during the neutropenic phase than it was during the period when neutrophils were at a normal level. Figure 13 compares reactions to a pyrogen derived from Pseudomonas aeruginosa (Piromen), typhoid vaccine, and streptokinase-streptodornase during the period of absolute neutropenia and at a time when normal numbers of neutrophils were present in the blood. These results can be interpreted as strong indication that the local reactions to Gram-negative bacterial endotoxin and the local reaction of bacterial allergy take place in the complete absence of circulating neutrophils.

Role of the Neutrophil in Development of Systemic Reactions to Gram-Negative Bacterial Endotoxins.—The intravenous injection of Gram-negative bacterial endotoxin in man results in a series of changes including an early decrease in numbers of circulating neutrophils and development of chills, severe headache, malaise, and fever. The fall in neutrophil count precedes the other events listed. In experimental animals a similar sequence is noted, in which the decrease in neutrophil count associated with development of adhesiveness of the neutrophils precedes the occurrence of fever,

development of shock, and death from the toxic effects of Gram-negative bacterial endotoxin. It has been shown that polymorphonuclear leukocytes are essential to the development of the Shwartzman and generalized Shwartzman reactions[72] (hemorrhagic necrosis produced by endotoxins), and it has recently been argued that the fever produced by endotoxin is a function, at least in part, of an endogenous pyrogen produced by polymorphonuclear leukocytes and other cells[66] injured by endotoxin. In order to determine whether the febrile effect of endotoxin or its toxic effects depend upon primary injury of polymorphonuclear leukocytes in man, the effect of administration of endotoxin on development of fever, chills, headache, and malaise during a period of complete neutropenia in our patient with cyclic neutropenia was studied. In addition, the effect of injection of endotoxin in two additional patients who were lacking in circulating neutrophils was investigated. The results of this study are summarized in Table 10, where it may be seen that fever, chills, headache, and malaise occur following injection of Gram-negative bacterial endotoxin in the complete absence of circulating neutrophils and that the fever occurring in the complete absence of circulating and tissue neutrophils was at least as high as that produced during the period when normal numbers of neutrophils were present in the blood. It must be concluded from this study that injury to neutrophils with liberation of endogenous pyrogen[67] plays no important role in the development of fever following the intravenous injection of pyrogens in man. These findings are oppositional to recent extrapolations made by Wood[84] from the experimental observations.

TABLE 10.—*Role of the Neutrophil in Development of Fever, Headache, and Toxic Reaction to Intravenous Injection of Gram-Negative Bacterial Endotoxin*

Patient	Headache 30 Min. After Injection of 20,000 TAB Organisms	Chills 1st Hr. After Injection	Temperature 2 Hr. After Injection, F
The patient, 15 yr.-old girl, cyclic neutropenia neutrophil count 0/cu. mm.	++++	++++	105.8
Neutrophil count 1,780/cu. mm.	+++	+++	104.8
5-yr.-old girl, chronic agranulocytosis, neutrophil count 60/cu. mm.	++	+++	105.2
Patient with acute agranulocytosis,* neutrophil count 0/cu. mm.	++	+++	103.2
12-yr.-old normal boy, neutrophil count 6,000/cu. mm.	++++	+++	104.8
6 yr.-old normal girl, neutrophil count 3,400/cu. mm.	+++	++	104.6

* Injection of typhoid-paratyphoid vaccine subcutaneously 750,000,000 organisms.

A hematological state similar to that observed in the neutropenic phase of cyclic neutropenia is produced in rabbits by the intravenous injection of mechlorethamine (nitrogen mustard) in large doses (1.5 to 1.75 mg. per kilogram). Following injection of this chemical there occurs an initial transient lymphopenia maximum 18 to 24 hours after injection and followed by an increase in lymphocyte count to near normal levels between 72 and 120 hours following injection of the mechlorethamine. Beginning 48 hours after injection of the mechlorethamine, the numbers of polymorphonuclear leukocytes declines so that between 72 and 144 hours following injection of the mechlorethamine virtually no polymorphonuclear leukocytes are present in the circulating blood and no evidence of either heteroplastic or homoplastic maturation of these cells in the bone marrow is observed. A study of acute-phase responses of rabbits, prepared by prior injection of mechlorethamine, to injection of Gram-negative bacterial endotoxins was carried out. The results, summarized in Table 11, demonstrate that the absence of polymorphonuclear leukocytes from the blood and bone marrow in the experimental situation does not interfere with the development of fever, production or liberation of C-reactive protein, and increase in mucoprotein concentration which occurs in normal rabbits following the intravenous injection of meningococcal or Serratia marcescens endotoxins.

TABLE 11.—*Response of Temperature, C-Reactive Protein, and Mucoprotein to Injection of Endotoxins in Neutropenic Rabbits* *

Group	Rabbits, No.	Mean Neutrophil Count Prior to Injection of Toxin, No./Cu. Mm.	Mean Neutrophil Count 24 Hr. After Injection of Toxin, No./Cu. Mm.	C-Reactive Protein In Serum * †		Mean Mucoprotein Tyrosine, Mg./100 Cc.		Rectal Temperature, F	
				Before Injection of Toxin	24 Hr. After Injection	Before Injection of Toxin	24 Hr. After Injection	Prior to Endotoxin	2 Hr. After Toxin
Neutropenic rabbits injected with meningococcal endotoxin	6	53.3	75.0	0/6	6/6	1.91	4.60	103.2	106.6
Neutropenic rabbits injected with P‡‡	6	146.0	62.2	0/6	5/6	2.20	5.70	103.6	106.3
Normal rabbits injected with meningococcal endotoxin	6	2,887.5	8,468.8	0/6	6/6	2.05	4.82	102.9	105.7
Normal rabbits injected with P‡‡	6	3,148.0	7,948.0	0/6	6/6	1.56	5.34	103.4	105.9

* Studied between 72 and 120 hours after injection mechlorethamine.
† Numerator indicates number of animals with C-protein in serum.

The observations in the experimental animals confirm and extend the results obtained in the study of the humans with pathological neutropenia and establish beyond reasonable doubt that the alterations in the acute-phase reactants observed in disease are independent of the functions of the polymorphonuclear leukocyte. The observations in the experimental animals further suggest that, in contradistinction to the recent arguments presented by Wood [66, 84] and Atkins et al.,[67,68] fever produced by Gram-negative endotoxin cannot be primarily a function of an endogenous pyrogen liberated from injured polymorphonuclear leukocytes. These observations are all the more significant since, thus far, it is only polymorphonuclear leukocytes which have yielded the "endogenous pyrogen" so attractive in current studies on the pathogenesis of fever.

Role of Neutrophils in Development of Refractoriness to Gram-Negative Bacterial Endotoxin.—Since evidence has been pre-

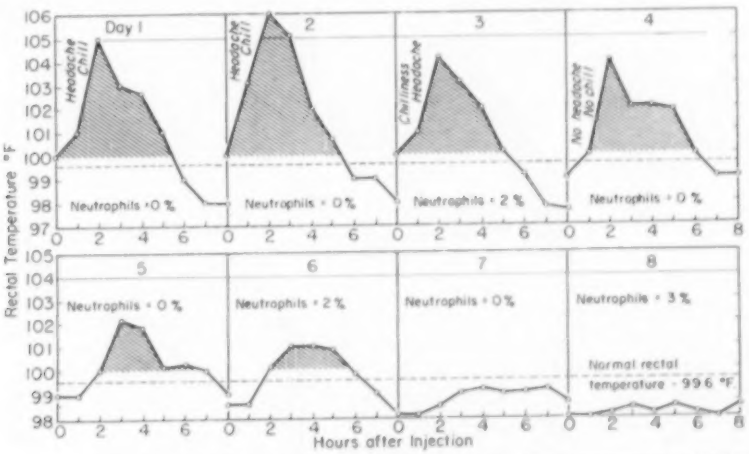


Fig. 14A.—Development of systemic reactions following daily intravenous injections of endotoxin (typhoid vaccine, 20,000,000 TAB organisms per day) in the patient during the neutropenic phase of the cycle (compare with Fig. 14B). These observations indicate that, contrary to conclusions of Atkins and Wood, an endogenous pyrogen derived from neutrophils is not essential to the production of fever or systemic reactions by endotoxin. It may further be noted that refractoriness to endotoxin develops equally well in the absence of neutrophils (Fig. 14A) as in their presence (Fig. 14B).

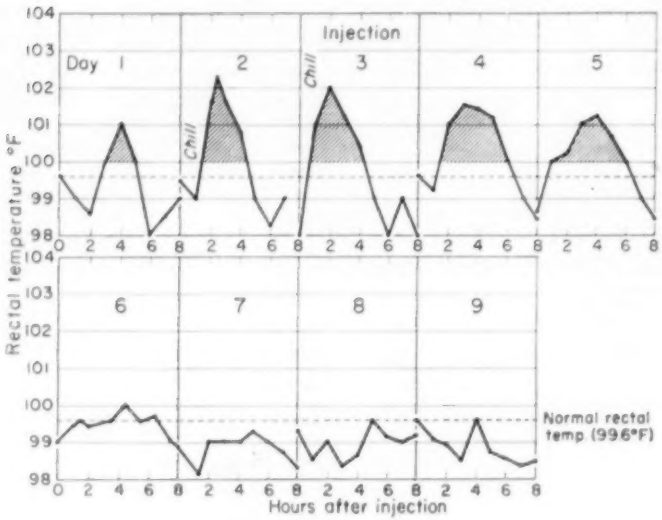


Fig. 14B. — Systemic reactions following daily intravenous injections of endotoxin (typhoid vaccine, 20,000,000 TAB organisms per day) in a normal girl who had normal numbers of neutrophils.

sented to indicate that the neutrophil is a prime target of endotoxins [73] and that polymorphonuclear leukocytes may aid in removal of endotoxin from the circulation,[74] it is conceivable that development of refractoriness to endotoxin is a function of a defense mechanism centering about the polymorphonuclear leukocytes. To test this hypothesis, repeated daily injections of endotoxin (typhoid-paratyphoid vaccine) were given to the patient with cyclic neutropenia during a prolonged neutropenic episode. These results (Figs. 14A and 14B) provide convincing evidence that refractoriness to endotoxin develops in the complete absence of neutrophils in the circulating blood or in the blood-forming tissues and consequently demonstrates that these cells are not essential to this defense reaction. These observations, together with convincing evidence provided through study of the agammaglobulinemic patients that circulating antibodies are not essential to the development of refractoriness to endotoxin,[75,76] serve to strengthen the hypothesis that in man, as in experimental animals, refractoriness to endotoxins is probably due in some way to alterations which take place in the function of the cells of the reticuloendothelial system.[77-79]

Role of the Neutrophil in Inflammatory Response.—The final experimental study was a comparison of the inflammatory response of the skin to mechanical injury and to application of antigen during the neutropenic and neutrophilic phase of the cyclic neutropenia. In these studies the method used was that described by Rebuck [80,81] for evaluating the morphological events comprising the inflammatory cycle in man. In essence, this method consists of scraping off the epidermis and, in some instances, applying an antigenic irritant as the inflammogenic agent. Analyzed in this way, the morphological events in the inflammatory cycle in normal persons consist of a stereotyped sequence of events with strikingly fixed time relationship which can be effectively evaluated by serial study of Romanovsky-stained preparations of cover slips which have been applied to the inflammatory lesions.

In the normal child the events of the inflammatory cycle, induced either by simple mechanical irritation or by antigenic stimulation, are as follows: There is an initial infiltration of histogenous mononuclear cells (histiocytes), beginning within 30 minutes; subsequent polymorphonuclear infiltration begins 30 minutes to 1 hour after

irritation and reaches a maximum during the next 3 to 4 hours. Beginning four to six hours after initiation of the inflammatory process, infiltration of small mononuclear cells indistinguishable from small, medium, and large circulating lymphocytes begins. During the subsequent 12 hours these cells undergo a dramatic cytomorphogenesis to become the hematogenous polyblasts and macrophages[81] of the acute inflammatory process. The consistent nature of the morphological sequence of events in the actue inflammatory process is striking and has recently provoked consideration.[82]

As part of a clinical and experimental study of the inflammatory process to be reported elsewhere,[83] the morphological mechanisms of the inflammatory response were studied in our patient with cyclic neutropenia. Separate studies were carried out at a time when neutrophils were completely absent from the circulation and again when nearly normal numbers of neutrophils were present in the circulating blood.

During the latter period the sequential cellular infiltration occurred as in the normal person. An abundant polymorphonuclear infiltration began one to two hours after the inflammation was initiated. Again, as in the normal person, the PMN infiltration was gradually replaced by the lymphocytic (small mononuclear cell) infiltration beginning at the six-hour stage. As in the normal person, the latter was transformed progressively into large mononuclear inflammatory phagocytes during the subsequent 12 hours.

The inflammatory process produced during the neutropenic phase of the cycle was strikingly different. Since no neutrophils were present in the peripheral blood, none were present in the early stages of the inflammatory process, and none appeared at any time during the entire 48 hours the inflammatory process was studied. This was, of course, the expected situation. More striking, however, was the observation that, although lymphocytes were present in the peripheral blood in normal numbers and monocytes were present in numbers greater than normal, gross deficiency of these elements as well as of polymorphonuclear cells characterized the inflammatory process in our patient during the neutropenic phase of the cycle. Because the lymphocytic invasion had been deficient, the 18- and 24-hour stages of the inflammatory cycle were featured by a striking deficiency of all hematogenous cells, including the hematogenous macrophages. Figure 15 compares the 18-hour stage of the inflammatory process observed when neutrophils were present in the blood (Fig. 15*A*) and when they were absent (Fig. 15*B*). Numerous hematogenous macrophages in all stages of development were present when the inflammation occurred during the neutrophilic phase of the cycle, whereas gross deficiency of the inflammatory response, with absence of lymphocytes and hematogenous macrophages, featured the inflammatory process when the neutrophils were absent from the serum. Similar observations were made when block biopsy specimens of the inflammatory lesions were obtained (Fig. 15*C* and *D*).

We interpret these observations as indication that the strikingly consistent morphological sequence of events in acute inflammation is a chain-like process with each stage dependent, to some extent at least, on the preceding one. Thus, when the polymorphonuclear leukocytes are absent the lymphocytic and mononuclear infiltration and subsequent development of hematogenous macrophages does not occur. It seems likely that the polymorphonuclear cells themselves or a product of their reaction with elements in the connective tissue contributes something to the inflammatory process which provokes the invasion of lymphocytes and conditions their ultimate transformation to macrophages in the acute inflammatory cycle. Indeed, this has been clearly shown to be the case in a subsequent experimental study based on this observation.[85]

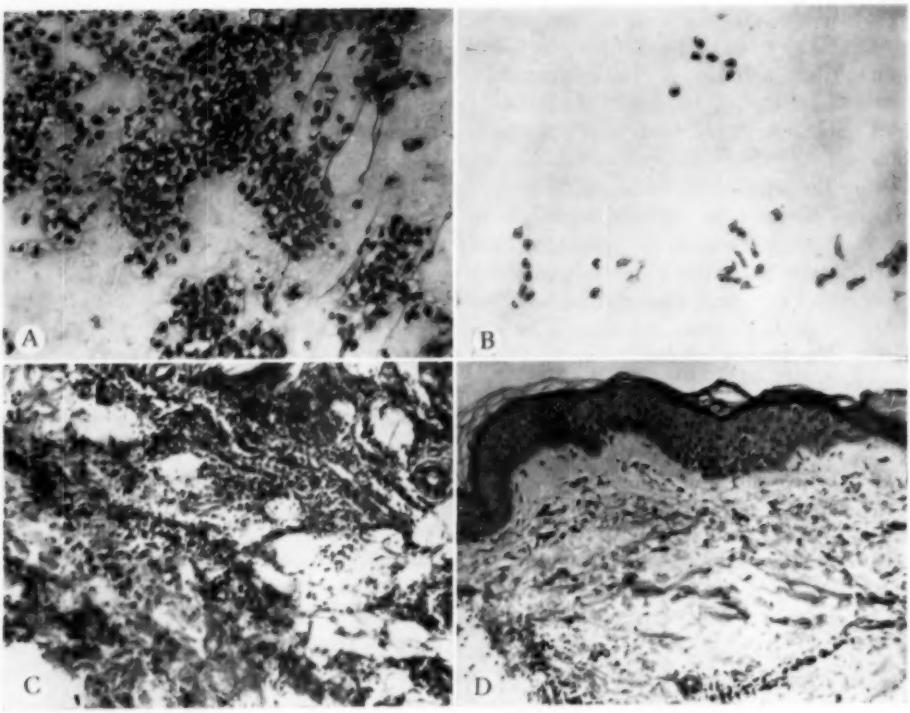


Fig. 15.—Inflammatory reaction of the skin in patient with cyclic neutropenia. Compared on this Figure is the cellular reaction of the skin during the neutrophilic phase of the inflammatory cycle, when near-normal numbers of neutrophils are present in the circulation (*A*), and that during the neutropenic phase of the cycle, when no neutrophils can be found in the blood (*B*). Shown is the 18-hour stage of the inflammatory cycle induced by skin irritation, Rebuck technique. Note that not only are neutrophils lacking in the exudate but that a comparable hematogenous mononuclear infiltration has also failed to occur. In Part *C* is illustrated the intense inflammatory response, both polymorphonuclear cells and round cells, observed in the skin biopsy specimen taken 24 hours after injection of typhoid vaccine intradermally during neutrophilic phase of the cycle; ×85. In contradistinction, Part *D* illustrates the lack of cellular exudate in the inflammatory reaction in a specimen taken 24 hours after injection of typhoid vaccine during the neutropenic phase of the cycle; ×85.

Therapy

In the past, only four methods of treatment have been reported as being helpful in the management of cyclic neutropenia. These include injections of staphylococcal vaccine, antibiotics, steroid hormones, and splenectomy. The first case described in 1910, by Leale,[1] was treated with staphylococcal vaccine when the patient was 3½ years of age. Although it was the author's impression that he had cured the disease with this therapy, Rutledge [2] restudied the patient 16 years later, at which time typical cyclic neutropenia with 21-day periodicity was again present. Antibiotics, of course, are helpful and often life-saving in treatment of the intercurrent pyogenic infections which frequently occur in these patients. For a time, because of the frequent necessity for penicillin therapy and the dramatic response produced, it was thought that penicillin not only cured the intercurrent infections but also brought about restoration of the neutrophil count and healing of the mucous membrane lesions in our patient.

It was finally established, however, that penicillin had no effect on the basic disease process but served only as therapy for the intercurrent bacterial infections. Continuous prophylactic antibiotic therapy, to our

knowledge, has been used only in our patient. She took continuous prophylactic penicillin in doses of 400,000 units per day for approximately two years. Although coincident with its use there was a decrease in frequency of severe life-threatening bacterial infections, the bacterial infections continued to occur and tended to be caused by the mutable micro-organisms (particularly staphylococci and Pseudomonas) resistant to antibiotics. Because of several very threatening experiences with these resistant infections in which it was necessary to take recourse to the newest antibiotics, we have reached the conclusion that continuous prophylactic antibiotic therapy is more likely to be harmful than beneficial and probably should not be used routinely in these patients.

Three recorded cases[11,21,22] have been treated with adrenal-pituitary hormones. One was given corticotropin intramuscularly, one was treated with crude adrenal cortical extract, and one was given a trial at separate times of both corticotropin and cortisone. In only one of these patients was a beneficial effect reported. In this patient, given a short period of treatment with corticotropin, a dramatic response was reported. Within a few days of the corticotropin treatment, however, splenectomy was performed, and the prolonged effects of corticotropin were not studied. From evaluation of these reports we were forced to the conclusion that in no prior instance had a convincing trial of adrenal-pituitary therapy been attempted in a clear-cut case of cyclic neutropenia and one could not ascertain from the literature whether or not these agents might be helpful in this disease. Consequently, we decided to give both corticotropin and cortisone a thorough trial as therapeutic agents in our patient.

Nine patients with cyclic neutropenia have been treated by splenectomy. In seven of these the authors reported varying degrees of symptomatic relief but the neutropenia continued to occur. Two patients were not improved by splenectomy.

Another possible therapeutic approach was suggested from study of the serial bone marrow samples taken on our patient. When we found periodic failure of maturation of the neutrophil precursors to be basic to the cyclic neutropenia, we postulated that neutrophilic maturation might be induced during the period of arrest by the parenteral injection of large numbers of mature neutrophils.

In attempting to inhibit the disease process in our patient, we have utilized each of these measures. Prophylactic and therapeutic antibiotic therapy have been given prolonged trial and have not altered the cyclic neutropenia or the cyclic appearance of oral ulcers, fever, and malaise so characteristic of the disease in our patient. Subsequently, parenteral injection of large numbers of mature leukocytes, steroid therapy, splenectomy, and staphylococcal vaccine have been utilized. In spite of all these measures the patient continues to have cyclic neutropenia every 21 days. Although steroid therapy had no effect on the cyclic neutropenia, it did result in abatement of clinical disease while large doses of hormone were being given. Also, after the return of symptomatology following discontinuation of steroid hormone, splenectomy resulted in rather striking clinical improvement without affecting the cyclic neutropenia. Because of these interesting observations in the treatment of this disease, our experiences are documented below.

To test the hypothesis that the neutrophilic arrest might be influenced by parenteral injection of neutrophils, the following experiment was performed. During the period when the patient's bone marrow showed maturation arrest she was given an intramuscular injection of approximately 1,000,000,000 leukocytes, of which 80% were neutrophils and 95% were viable (trypan blue technique). Serial cell counts of bone marrow and peripheral blood showed no response to the injection. Indeed, the rising concentration of bone marrow promyelocytes occurred as usual and the neutropenia

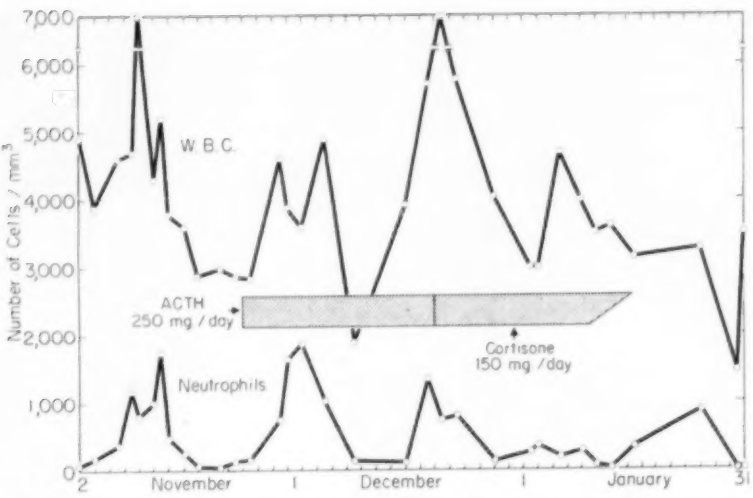


Fig. 16.—Effect of steroid treatment on cyclic neutropenia. Treatment with neither corticotropin nor cortisone prevented the occurrence of the cyclic neutropenia, although treatment with either drug prevented the development of malaise, fever, and oral ulcers which regularly accompanied the neutropenic phase of the cyclic process in the absence of treatment.

persisted just as long as in the usual cycle. Apparently, then, failure of maturation of the neutrophils cannot be relieved by administration of normal mature neutrophils.

Steroid therapy was given over a period of seven weeks. First corticotropin was given for a period of three and one-half weeks, and then cortisone was given for a similar period. Following completion of the trial the cortisone dose was gradually reduced over a one-week period. The results of the therapy were evaluated by recording serial neutrophil counts and by comparing the differential cell counts of serial bone marrow specimens taken at this time.

As is shown in Figure 16, neither form of steroid therapy prevented development of profound neutropenia, and the cyclic blood dyscrasia continued. During cortisone treatment, however, the duration of the cycle was somewhat shorter (16 days) than had previously been the case (21 days). However, following discontinuation of the steroid treatment the child returned to the usual 21-day periodicity.

The most striking alteration occurred in the clinical symptomatology. During the entire seven-week period of adrenal steroid therapy the patient was free of oral ulcers, fever, and malaise, which previously were regular concomitants of the neutropenic phase of her cycle. Figure 17 compares the appearance of the patient's mouth during a period of neutropenia prior to and during steroid treatment. In Figure 11 the elimination of bone marrow plasmocytosis and hypergammaglobulinemia as a consequence of steroid therapy is illustrated. Following discontinuation of the steroid hormone the characteristic symptomatology of the disease recurred, and for a three-month period oral ulcers, fever, and severe malaise recurred every 21 days in association with the disappearance of neutrophils from the circulating blood.

This observation, as well as the reports of clinical improvement following splenectomy, encouraged us to try splenectomy in our patient in an effort both to alter the severity of the neutropenia and to affect the clinical course of this disease.

Effect of Splenectomy on the Course of Cyclic Neutropenia

Subsequent to the period of adrenal steroid treatment the patient was followed in the hospital for three months. During this time her disease

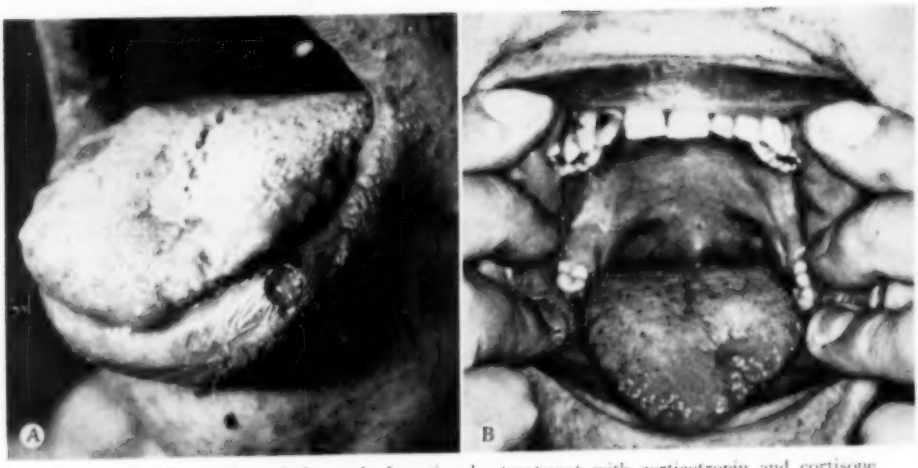


Fig. 17.—Prevention of the oral ulceration by treatment with corticotropin and cortisone. The characteristic punched-out ulcers regularly occurring in the oral mucous membranes during the neutropenic phase of the cycle are illustrated (*A*), as is their absence during steroid hormone therapy (*B*). The lesion on the left side of the tongue is an old scar.

expressed its typical clinical picture with 21-day cycles featured by complete disappearance of neutrophils from the circulation, development of oral ulcers, fever, and malaise. As before the steroid treatment had been tried, these episodes lasted approximately one week, three to four days of which the patient spent in bed because of marked malaise.

On March 30, 1956, at a time when the neutrophils were beginning to appear in the peripheral blood, splenectomy was performed. A somewhat enlarged spleen (250 gm.) showing normal gross appearance was removed. Microscopic examination revealed only relatively prominent sinusoids and littoral cells, which were easily visualized. Although neutrophils were present in the red pulp, their number was markedly lower than that present in the normal spleen. The lymphoid follicles appeared normal. Sections of a lymph node removed with the spleen showed only slight reticular hyperplasia. Extensive bacteriologic cultures, animal inoculations, and search for a virus agent in the spleen and lymph nodes were negative.

The child recovered from the operation without event. She showed the prompt leukocyte and platelet rise in the peripheral blood which usually follow splenectomy. Since that time she has been followed continuously over a 12-month period. Although there has been no interruption whatever of her cyclic neutropenia (Fig. 18), during a six-month period following splenectomy she had no oral ulcers or malaise. This improvement represented a dramatic change in the clinical expression of her illness. However, during the last six months of this period she has had eight episodes of neutropenia associated with the usual fever, malaise, ulceration of the oral mucosa, and skin ulcers which characterized her clinical disease prior to splenectomy.

It must be concluded from these observations that, as in other reported cases, splenectomy had no significant effect on the cyclic neutropenia but did induce a temporary remission of the clinical manifestations associated with the neutropenic episodes. After a six-month remission, however, an exacerbation occurred and the entire clinical and laboratory syndrome returned as it had been prior to splenectomy.

Effect of Staphylococcal Vaccine

The final effort at treatment of the cyclic neutropenia has been an attempt to confirm the observation of Leale [1] that staphylococcal vaccine resulted in abatement of clinical symptomatology and disappearance of cyclic neutropenia. Three months after splenectomy a series of injections of staphylococcal vaccine (Lilly) was started. In this series the injections given were initially 0.25 cc. two times per week, and increasing doses of vaccine were given each week until after four weeks the patient was receiving 1 cc. of the crude vaccine per week. This treatment, continued for four months, resulted

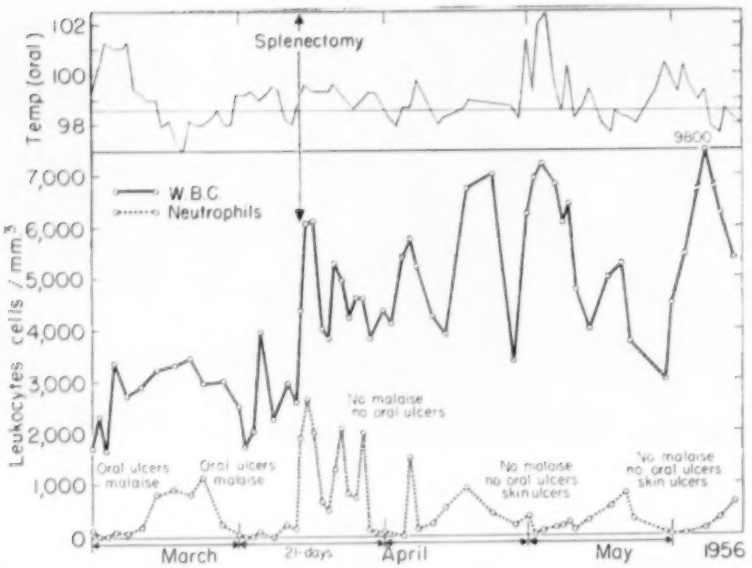


Fig. 18.—Effect of splenectomy on symptoms, fever, leukocyte count, and neutrophil count in patient with cyclic neutropenia. Removal of the spleen had no demonstrable effect on the episodes of neutropenia. However, for a period of six months following removal of the spleen no malaise or oral ulcers occurred. Subsequently, the entire clinical picture returned, and no lasting demonstrable difference in the clinical or laboratory features of the disease could be attributed to splenectomy.

in no demonstrable change in the neutrophil count, and the cyclic neutropenia continued uninterrupted. Also, no alteration in the clinical syndrome could be attributed to the vaccine treatment.

Summary of Studies and Conclusions

With use of a variety of techniques, an extensive search has been made in an effort to determine the etiologic mechanism of this disease. Although the exact etiology has not been discovered, much has been learned that serves better to define the disease process.

(a) Transfusion of the neutropenic patient's blood into a patient with myelogenous leukemia and infusion of large amounts of the patient's serum and blood (neutropenic phase) into a leukemic child, two normal children, and a child with mild leukocytosis did not produce fever, oral ulcers, skin ulcers, or leukopenia.

(b) Even though three different methods of demonstration were used, no leukoagglutinins could be found at any time during the cycles of neutropenia. Consequent to these findings we conclude that there is no immunologic basis for this disease.

The patient's serum was evaluated for the presence of a toxic factor which might injure the polymorphonuclear leukocytes, account for the "toxic" appearance of the cells which we have observed, and result in the cyclic neutropenia. The toxic factor was sought by studying the migration and phagocytic ability of these cells. The results demonstrate that the patient's serum taken at various phases of the neutrophil cycle did not alter the functions of normal neutrophils. In addition, the patient's own neutrophils, when present in the blood, were functionally normal as evaluated by these methods.

Serial bone marrow specimens taken during a single cycle and numerous bone marrow specimens taken during the various phases of other cycles revealed cyclic fluctuations of the concentrations of reticulum

cells, myeloblasts, promyelocytes, neutrophilic myelocytes, and neutrophilic metamyelocytes which appeared to be basic to the fluctuations of the neutrophil count in the peripheral blood. These observations indicated that the cyclic neutropenia is due to a cyclic arrest of production of the entire neutrophil series. In addition, the concentration of promyelocytes was regularly greater than normal and rose to highest concentration just prior to return of neutrophils in the peripheral blood. It is postulated that two morphologically definable defects underlie the hematological abnormality observed in this patient: (*a*) periodic failure of heteroplastic maturation of neutrophils from primative reticulum cells; (*b*) defect in maturation at the promyelocyte stage, resulting in accumulation of promyelocytes in the bone marrow, which becomes especially notable when heteroplastic maturation is proceeding normally.

An evaluation of the patient's hormone status revealed that her menstrual cycles were not related to the periodic fluctuations in neutrophil count. Measurements of urinary excretion of FSH and 17-ketosteroids, blood corticotropin concentration, 17-hydroxycorticosteroid concentration, and adrenal response to corticotropin stimulation were all within normal limits and did not fluctuate during the neutrophil cycle. Although the studies conducted did not demonstrate any hormonal abnormality in this patient, the possibility that cyclic neutropenia is caused by disturbance in hormonal function involving central control mechanisms has not been eliminated.

Although the patient was found to have mild hypergammaglobulinemia and plasmacytosis of the bone marrow, we demonstrated that her neutrophil cycle was independent of fluctuations in these components.

Serial determinations of acute-phase reactants during a complete neutrophil cycle did not reveal any cyclic abnormalities. Except for the sedimentation rate, which constantly showed moderate elevation (probably because of the high serum γ-globulin), the acute-phase reactants studied (fibrinogen, heparin-precipitable factor, mucoprotein, and C-reactive protein) were within normal limits. Since the acute-phase reactions are regularly abnormal during infection, these observations were interpreted as evidence against a chronic or recurrent infectious process being the cause of her disease.

Properdin determinations done during the neutropenic and neutrophilic phases of the patient's cycle were normal and showed no significant variation at the various phases of the cycle. This is evidence that the production or liberation of properdin is not dependent upon the presence of or formation of neutrophils.

Taking advantage of the cyclic fluctuation in neutrophil count, an attempt was made to evaluate the role of the neutrophil in several biologic processes. The results of these studies are as follows:

(*a*) The patient's antibody responses to typhoid paratyphoid vaccine, diphtheria toxoid, mumps antigen, and poliovirus during a period of persistent neutropenia were normal. In addition, similar data were obtained on a patient with agranulocytosis and aplastic anemia. These data demonstrate that neutrophils do not perform a function essential to the production of circulating antibodies.

(*b*) The production of acute-phase serological reactions (C-reactive protein, mucoprotein, and elevated erythrocyte sedimentation rate, is not dependent on the presence of neutrophils, as demonstrated in our patient with cyclic neutropenia during a period of neutropenia, in a patient with agranulocytosis, and in rabbits made neutropenic by the injection of mechlorethamine.

(*c*) The delayed type of skin reactions to the intradermal injection of Piromen, streptokinase-streptodornase, and tetanus-paratyphoid A-paratyphoid B (TAB) vaccine were compared during the neutrophilic and neutropenic phases of the patient's cy-

cle, and no gross difference in response occurred at these times.

(*d*) Comparison of the capacity to develop chills, fever, headache, and malaise following intravenous injection of Gram-negative bacterial endotoxin during the neutropenic and neutrophilic phase of the patient's cycle revealed no differences attributable to the neutrophilic fluctuation. These observations, together with supporting data from animal experimentation, provoke the conclusion that in neither man nor experimental animals does endogenous pyrogen derived from polymorphonuclear leukocytes play an important role in the reaction produced by these toxins.

(*e*) Our patient developed a virtually complete refractoriness to endotoxin during a period when neutrophils were completely absent from the blood and bone marrow, indicating that neutrophils are not essential to the development of a high degree of refractoriness to the toxic and pyrogenic effects of Gram-negative bacterial endotoxins.

(*f*) Comparison of the inflammatory cycle induced by mechanical or antigenic irritation of the skin during the peaks and the depths of the neutrophilic cycle revealed that the inflammatory response was markedly abnormal when neutrophilic infiltration did not occur. In addition to the absence of neutrophils from the inflammatory exudate, the lymphocytic (or mononuclear) exudation did not occur and hematogenous macrophages did not develop. This observation suggests that the neutrophilic infiltration in some way conditions the lymphocytic invasion and consequent macrophage accumulation in acute inflammation.

Attempts at treatment included prophylactic and therapeutic administration of antibiotics, parenteral injection of normal leukocytes during the neutropenic phase of the cycle, a thorough trial of both corticotropin and cortisone in large dosage, splenectomy, and staphylococcal vaccine therapy. None of these measures resulted in significant alteration of the cyclic neutropenia, and none of the measures, save corticotropin, cortisone, and splenectomy, altered the clinical symptomatology. Treatment with either corticotropin or cortisone resulted in abatement of the cyclic appearance of fever, oral and skin ulcers, and severe malaise which accompanied the cyclic neutropenia. However, the symptomatology returned as soon as the hormone therapy was discontinued. Although splenectomy had no demonstrable effect on the cyclic neutropenia, the associated clinical illness was absent for six months after splenectomy, only to return in its characteristic form.

Summary

The world medical literature on the rare disease cyclic neutropenia is reviewed, and studies on the nature of this disorder performed by others are evaluated.

A case of cyclic neutropenia in a 15-year-old girl who has been intensively studied over an 11-year period is presented. The basis of the cyclic neutropenia appears to be cyclic failure of neutrophilic maturation.

Although extensively sought, no evidence of the appearance of a factor injurious to or destructive of neutrophils was found in the peripheral blood at any time. Study of the patient's growth and development, menstruation, and specific pituitary-adrenal-gonadal function revealed no evidence of a hormonal factor responsible for this syndrome.

Extensive studies of neutrophilic function based on interpretation of this experiment of nature are presented.

The results of treatment with adrenal steroids, splenectomy, staphylococcal vaccine, and injection of normal leukocytes are reported.

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The Treatment of Iron-Deficiency Anemia in Children with Iron-Dextran

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A. Introduction

During the past several years there has been a recrudescence of interest in the metabolism of iron together with a new interest in the treatment of iron-deficiency anemia. This has been brought about because of the availability of new investigative tools and techniques and by the manufacture of new and effective parenteral iron products.

It is the purpose of this paper to report on a clinical trial of an iron-dextran complex for intramuscular administration (Imferon), used in the treatment of iron-deficiency anemia in infants and children.

B. Materials and Methods

Sixty-five infants and children are included in this study. The ages range from 2 months to 7 years, with a mean age of 14 months. There are 37 boys and 28 girls. Of these, 51 are Caucasians, 6 are Spanish-Americans, 7 are Negroes, and 1 is Oriental. Twenty-five of these patients had birth weights under 2500 gm. In general, criteria used to select these children were based on the level of hemoglobin (below 8.5 gm/100 ml. of blood), with the anemia being hypochromic and microcytic.

The pretreatment work-up consisted of a complete history with emphasis on dietary, maternal and birth history; physical examination, and the following laboratory procedures: urinalysis, red blood cell count, white blood cell count, hemoglobin determination, differential count, microhematocrit determination, and reticulocyte count. After the diagnosis was established, the patients were given the following therapeutic regimen: (*a*) regular diet for age; (*b*) supplementary daily vitamins, particularly A (1500 to 2500 units) and D (400 to 800 units) and ascorbic acid (vitamin C) (75 mg.).

Although it is well recognized that no formula will give an accurate account of the patient's iron deficiency, two formulas were used in this preliminary study as a rough guide to the dosage of iron to be given each patient. The formulas used are as follows:

Mg. elemental Fe=B. W.×[16−(Hb.×1.3)]

or

Mg. elemental Fe=B. W.×1.5(13−Hb.)

Where B. W. is weight in pounds and Hb. is the current value of the patient's hemoglobin in grams/100 ml. of blood.

Inpatients received consecutive daily doses, and the outpatients received consecutive weekly doses of iron-dextran complex. A maximum single dose of 200 mg. was never exceeded, and there was never given more than 100 mg. per injection site. The iron-dextran complex was administered by deep intramuscular injection in the upper outer quadrant of the buttock.

Erythrocyte count, leukocyte count, hemoglobin determination performed by the Haden-Hausser method, microhematocrit determination (modified Guest-Silver), reticulocyte count using the dry method, and a differential count with emphasis on the erythrocyte morphology were performed before the treatment was instituted and were repeated weekly thereafter for the first month and monthly for the next two months. At each outpatient visit the patient was examined by the same person and an interval history was obtained from the mother. Toward the end of the study, some patients were examined on a monthly basis. As a guide to the efficiency of the treatment, the following normal hemoglobin levels for the various ages have been used[a]: 6 to 11 months, 11.8 gm.; 1 year, 11.2 gm.; 2 years, 11.5 gm.; 3 years, 12.5 gm. per 100 ml. of blood.

C. Clinical Findings

Patients were seen at Children's Hospital as in-patients or as out-patients with a history or chief complaint of pallor, lassitude, and/or infection, chiefly respiratory. Some

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Case Studies

Case No.	Age		Sex	Race	Iron-Dextran, Mg.	Hemoglobin, Gm./100 Ml.						
	Yr.	Mo.				Initial	1st Wk.	2d Wk.	3d Wk.	4th Wk.	2d Mo.	3d Mo.
1		8	F	W	200	4.0	..	9.25	9.5	..	..	..
2	1	3	M	SA	200	5.5	7.5	8.75	10.5	10.5	12.5	..
3	2	3	F	W	200	5.25	10.0	10.5	..	12.5	13.5	12.0
4	1		F	W	150	7.00	8.5	11.0	11.5	11.0	12.5	13.0
5	1		F	W	200	5.75	8.5	10.0	11.0	12.0	12.5	11.0
6		9	F	W	100	8.5	9.0	10.5	..	10.0	9.5	11.0
7		11	M	W	100	6.5	9.5	..	..	11.0	..	..
8	1	2	F	W	150	8.0	11.0	10.5	11.5	11.5	12.5	13.0
9	1	8	M	W	200	6.0	9.75	10.5	11.5	11.5	12.5	..
10	1	6	M	W	250	6.0	7.75	10.5	11.0	11.5	11.0	12.0
11		5	F	W	150	7.75	11.0	10.5	12.0	12.0	12.0	12.5
12		8	M	W	200	4.25	6.5	10.0	10.5	10.5	12.5	13.5
13		11	M	W	100	9.0	9.0	11.0	..	10.5	12.0	..
14		7	F	W	150	5.25	7.75	..	9.0	10.0	10.5	10.0
15		7	F	W	200	4.25	10.0	10.5	11.0	12.5	13.5	12.0
16	1	1	M	W	250	5.5	7.75	9.0	10.0	11.0	13.0	11.0
17	1	9	M	W	250	6.0	8.0	8.75	10.5	10.5	11.5	11.5
18	1	2	M	N	150	7.5	7.5	8.5	9.0	..	10.0	..
19	1	8	F	SA	150	8.25	9.25	..	..	10.5	..	..
20		7	M	SA	150	4.75	9.5	9.75	12.5	..	..	..
21	1	9	F	SA	200	6.5	9.25	11.0	12.0	12.5	12.5	12.0
22	1	2	F	W	200	5.0	7.75	10.0	11.0	11.0	13.0	10.5
23	2		M	W	200	6.5	8.25	9.5	11.0	10.5	11.0	12.0
24		11	F	SA	200	4.75	7.75	..	..	12.5	11.0	11.0
25		9	M	W	100	8.0	9.5	11.0	10.5	11.0	12.0	12.5
26		11	M	W	150	6.0	8.5	10.5	11.0	12.5	12.0	12.5
27	1		F	W	200	4.0	7.5	9.25	10.0	10.5	10.0	12.5
28	1	1	M	W	150	7.75	10.0	..	..	..	12.0	12.0
29	1	1	M	N	150	8.75	9.25	10.5	..	..	..	11.5
30	1		M	N	150	7.5	8.5	9.25	10.0	10.0	10.5	11.5
31		9	M	W	100	8.25	10.5	10.0	11.5	11.0	11.0	10.0
32	1	5	M	W	150	6.5	9.5	9.5	12.0	..	..	..
33		9	M	W	150	7.25	9.5	..	..	..	..	..
34	1	10	F	W	150	8.5	9.5	11.0	10.5	11.0	11.5	12.0
35		11	M	W	200	5.5	..	..	..	11.0	11.0	..
36	1	11	M	W	250	5.25	8.25	10.0	11.5	11.0	12.0	12.5
37	1		M	N	150	8.0	9.5	11.0	10.5	10.5	12.5	..
38 *		8	M	W	350 (200 & 150)	4.75	7.75	..	9.5	8.75	8.25	7.0
39		5	F	SA	100	7.5	9.5	..	..	..	..	..
40 †		10	F	W	200	4.75	..	..	..	7.25	7.75	10.5
41		11	M	W	100	8.5	9.5	9.0	10.0	..	..	..
42	1	1	F	W	200	5.5	..	..	10.0	10.5	11.5	11.5
43	1		M	W	200	7.0	9.25	10.5	11.0	12.0	..	..
44	1	1	M	W	150	7.5	9.5	12.0	12.0	11.0	10.0	12.5
45	1		F	W	150	8.0	9.0	10.5	..	..	..	11.5
46		7	F	N	100	8.5	..	..	..	..	10.5	11.5
47	1	4	M	W	150	8.0	11.5	11.0	12.5	12.0	12.5	..
48	1	1	F	W	200	6.5	..	10.0	..	..	..	..
49 ‡		2	F	W	100	8.0	..	..	9.0	8.5	10.0	10.0
					100							
50	7		M	W	100							
51	5	6	M	O	400	5.0	8.25	9.5	9.75	10.0	10.0	10.5
52		7	M	N	200	8.0	..	..	..	10.5	..	..
53	1		M	N	100	8.0	10.5	10.5	11.0	12.0	11.0	..
54	1		M	W	200	5.25	..	..	..	..	..	10.5
55		7	F	W	150	7.0	..	..	..	11.5	10.5	11.0
56 *		7	F	W	100	7.5	..	..	..	10.5	..	11.0
					200	4.25	..	..	..	9.75	8.5	6.0
57	1	2	F	W	200							
58 ‡		2	F	W	150	6.25	..	..	..	13.0	..	12.5
59	1	6	M	W	100	9.5	10.0	..	..	10.5	..	11.5
60 *		9	M	W	300	3.25	..	..	..	9.75	10.0	11.5
					100	6.75	9.5	..	..	10.5	..	8.0
61		10	M	W	150							
62 ‡		3	M	W	250	3.25	7.5	9.0	10.5	..	..	..
63	3		F	W	100	8.75	10.5	..	9.5			
64		11	M	W	300	4.75	6.5	7.0	9.5	10.5	11.0	..
65		9	F	W	250	3.75	7.5	9.75	10.5	..	..	13.6
					100	8.5	..	..	..	11.0	..	11.0

* Patients who relapsed and who were treated with a second course of iron-dextran complex.
† Patient treated with folic acid during the second- and third-month follow-up.
‡ Premature infants.

patients had combined complaints. Pallor was found in 39 patients; respiratory infection, in 20; gastrointestinal infection, in 3, and miscellaneous complaints, in 15.

In general, all patients had a poor dietary history and subsisted almost entirely on milk. Blood loss played no part in the etiology of the iron deficiency. Occasionally the mother was said to have been anemic during pregnancy. The family history was otherwise not significant.

On physical examination significant findings other than pallor were hemic murmur (32 cases), splenomegaly (17 cases), cranial bruit (15 cases), and hepatomegaly (12 cases).

Case Material

The following cases are described in detail. They represent examples of iron deficiency and some of the complicating problems encountered. A summary of all the cases studied may be seen in the Table.

CASE 3.—*Cardiac Failure and Anemia*

A 27-month-old white girl was admitted in congestive failure with a clinical diagnosis of congenital pulmonic stenosis. For the previous year she had refused most solids and had existed principally on "milk and crackers." Her hemoglobin value was 5.25 gm/100 cc., and her red blood cell count was 2,670,000 per cubic millimeter. She weighed 26½ lb. (12 kg.) and was 81 cm. in body length. Extensive edema gave her a nephrotic appearance.

In response to digitalis and diuretic therapy she lost 2½ lb. (1. kg.) of edema fluid and was relieved of the congestive failure. Because of the serious risk of circulatory overload in correcting her anemia with whole blood transfusion, she was given 200 mg. of iron-dextran complex intramuscularly, 100 mg. in each buttock. Five days later her hemoglobin had risen to 10 gm/100 cc., and the RBC, to 3,720,000 per cubic millimeter, and reticulocytosis of 14.7% was present. Cardiac catheterization on the eighth hospital day confirmed the diagnostic impression of valvular pulmonic stenosis. On the following day, under general and hypothermic anesthesia, pulmonary valvuloplasty was performed. Her subsequent course was uncomplicated, and she was dismissed home on the 21st hospital day with a hemoglobin value of 10.5 gm/100 cc. and a red blood cell count of 3,680,000 per cubic millimeter.

Since dismissal she has shown further clinical and hematologic improvement. At 30 months of age she had a hemoglobin of 12.0 gm/100 cc. and RBC of 4,020,000 per cubic millimeter. Serial x-rays have shown marked diminution in heart size. She has been taking a more adequate diet and has made a substantial nutritional gain. When last seen, at the age of 3 years, her hemoglobin was 12.5 gm/100 cc. and her red cell count numbered 4,080,000 per cubic millimeter.

Comment.—This girl was in marked congestive failure as a result of a congenital cardiac lesion that limited the pulmonary blood flow and was doubly handicapped by the severe iron-deficiency anemia. Rapid correction of the anemia was safely accomplished with intramuscular administration of iron, and the hazards of cardiac overload were circumvented.

CASE 6.—*Anemia Related to Chronic Infection*

A 9-month-old white girl was admitted with bronchitis. She had a history of frequent bouts of bronchitis since the age of 2 months. Her diet had consisted of milk, cereal, and fruit. She was small for her age and noticeably pale. Her initial hemoglobin value was 8.5 gm/100 cc., with 4,010,000 RBC per cubic millimeter, and microcytosis and hypochromia were evident in the peripheral blood smear. The bronchitis improved with antibiotic treatment, and prior to her discharge she received 100 mg. of iron-dextran complex intramuscularly.

She had two subsequent bouts of bronchitis after going home, and at the end of two months her hemoglobin had risen to only 9.5 gm/100 cc. The red blood cells totaled 3,920,000 per cubic millimeter; however, in the stained smear the cells were now normocytic and normochromic. She remained free of infection during the third month; her diet improved, and at the age of one year her hemoglobin was 11.0 gm/100 cc. and the erythrocyte count was 4,830,000 per cubic millimeter.

Comment.—This infant had a moderate iron-deficiency anemia, and during the recurring infections she failed to respond dramatically to intramuscular administration of iron. This is comparable to the result one expects when iron is given orally. Nevertheless, there was evidence of improvement in the appearance of the red blood cells, with gain in hemoglobin content. When the infections were at last brought under control, there was prompt rise in total red cells and a further improvement in the hemoglobin value.

CASE 12.—*Severe Uncomplicated Nutritional Anemia*

An 8-month-old white boy was seen as an outpatient because of persistent pallor. He was one of twins, having weighed 3 lb. 3 oz. (1530 gm.) at birth. His twin brother had died with respiratory distress at the age of 36 hours. At 8 months of age this infant was living on 40 oz. (1200 cc.) of homogenized milk per day, but his solid food intake was almost nil. He was markedly pale and had a cranial bruit and a hemic systolic cardiac murmur. His initial hemoglobin value was 4.5 gm/100 cc., and his red cell count was 2,200,000 per cubic millimeter. He weighed 16 lb. (7260 gm.) and measured 67 cm. in length.

On the basis of calculated iron deficit he was given 250 mg. of iron dextran complex in divided doses at weekly intervals as an outpatient. Two weeks after the initial injection the murmur and the bruit had disappeared and his appetite and color had improved dramatically. His hemoglobin

had risen to 10.0 gm/100 cc., and the red blood cell count was 3,860,000 per cubic millimeter. The remainder of his observed course was uneventful, and at the age of 11 months his hemoglobin content was 12.5 gm/100 cc. and his red blood cells totaled 4,290,000 per cubic millimeter.

Comment.—This boy was a premature infant who had always been pale and had a deficient intake of iron. A cranial bruit noted on physical examination allowed a clinical estimate of a hemoglobin level less than 5 gm/100 cc. His dramatic response to Imferon emphasized the safety of such treatment in the low-hemoglobin group. The advantages over whole blood transfusions in such an instance are obvious.

CASE 11.—*Moderate Uncomplicated Nutritional Anemia*

A 5-month-old white girl was admitted with laryngotracheobronchitis. Birth weight was 4 lb. 13 oz. (2310 gm.), and the early months were unremarkable except for the character of her diet. Foods containing good sources of iron were refused, but milk was ingested in such quantities that when she entered the hospital at 5 months of age she was drinking 72 oz. (2160 cc.) of homogenized milk per day. She weighed 18 lb. (8165 gm.) and measured 68 cm. in length. In addition to the respiratory tract findings, she exhibited pallor, a hemic murmur, and obesity. The initial hemoglobin value was 7.75 gm/100 cc., and RBC numbered 2,600,000 per cubic millimeter. The peripheral smear revealed marked microcytosis and hypochromia.

She was given 150 mg. of iron-dextran complex, and one week later her hemoglobin had risen to 11.0 gm/100 cc. and the red blood cells were 3,770,000 per cubic millimeter, with striking improvement in the cellular appearance. The hemic murmur present when she was first seen had disappeared. Her milk intake was reduced to 24 oz. (720 cc.) per day, and the intake of solid foods was proportionally increased. By the third month under observation the hemoglobin value was 12.5 gm/100 cc. and the red blood cell count was 4,310,000 per cubic millimeter.

Comment.—This case, a relatively mild anemia in this series, responded well to intramuscular administration of iron. With correction of the diet, improvement was maintained.

CASE 49.—*Anemia of Prematurity*

A 2 lb. 12 oz. (1020 gm.) premature girl was admitted at three days of age in relatively good condition. Her hemoglobin on admission was 15 gm/100 cc., and the red blood cell count, 4,720,000 per cubic millimeter. She did well with routine premature infant care, and at 2 months of age she weighed 3 lb. 11 oz. (1770 gm.). The hemoglobin at that time had fallen to 8.0 gm/100 cc., and the red blood cell count, to 2,520,000 per cubic millimeter; reticulocytes numbered 1.6%. At that time she was given an injection of 100 mg. of iron-dextran complex, and four days later her reticulocytes had increased to 13%, with no essential change in the remainder of the blood count. She was dismissed from the hospital at the age of 2½ months with a weight of 4 lb. 11 oz. (2250 gm.). A blood count was not done at that time. At 4 months of age her hemoglobin value was 10.0 gm/100 cc. and the RBC numbered 3,670,000 per cubic millimeter. She was then given another 100 mg. of iron-dextran complex, and when she was found to have essentially the same peripheral blood picture at 5 months of age another 100 mg. of iron-dextran complex was administered. She was last seen at the age of 7 months, weighing 15 lb. (7200 gm.) and having grown proportionately in length. Her hemoglobin was 12 gm/100 cc., and her red blood cell count was 3,880,000 cubic millimeter. Diet had been supervised and was well balanced. She had experienced no significant illnesses, and developmental survey revealed acceptable behavior for her age.

Comment.—The usual premature infant who does well reaches a low level of the erythrocytes and hemoglobin at approximately 9 weeks of age. Following this physiologic decline the rebuilding phase ensues, during which the hemoglobin lags behind the red blood cell production because of inadequate iron for hemoglobin synthesis. Daily oral iron can be given to supplement the diet and has been generally successful. Iron-dextran complex was used for this premature infant, and the utilization of the injected iron appears to have been excellent, since the initial rise was accomplished without any significant iron-containing foods in the diet. No evidence developed to indicate that the iron was harmful, either locally or systematically, in the doses given.

Comment

From the foregoing data it may be concluded that iron-dextran complex is a safe and rapidly effective form of iron for use in the treatment of iron-deficiency anemia. In the case material presented, failure to

get a rapid response indicated an additional deficiency or some complicating disease, i. e., folic acid deficiency or chronic infection. Even in the cases suffering from infections the microcytic hypochromic anemia was converted to a normochromic anemia of infection. The choice of daily dosage versus weekly dosage had no demonstrable effect on the speed or amount of hemoglobin formation. In considering the initial physical findings, one may conclude that the lower the hemoglobin level the greater the chance of finding a hemic systolic murmur or enlarged liver. In general, a cranial bruit signifies a hemoglobin level below 5.0 gm. per 100 cc. of blood.

A review of the differential white blood cell counts on these patients revealed an eosinophil level of 5% or higher (range, 5% to 28%) in 36 cases (55%). In 12 of these cases there was eosinophilia before therapy was started, and 24 cases developed the eosinophilia after the therapy. None of the usual causes of eosinophilia could be demonstrated. The exact significance of this observation is not clear.

Paralleling the hematologic response to the therapy, an improvement in activity, color, and appetite was noted in virtually all of the patients. The hemic murmurs disappeared in most of the patients. Those persisting were assumed to be innocent in nature. The cranial bruits disappeared and the liver enlargement receded as the hemoglobin level increased.

Of the 65 cases followed (16 for more than three months) only 4 cases developed a secondary iron-deficiency anemia after an initial favorable response. All such cases responded to a second course of iron therapy. The cause of the relapse is considered to be on a basis of rapid growth and expanding hemoglobin mass and/or reversion to a "milk diet."

This study failed to reveal any adverse local or systemic effects of the intramuscularly injected iron-dextran complex used. Pain at the site of the injection was interpreted as being comparable to that of a penicillin injection of equal volume. A few skin stains were encountered at the injection site, but the pigmentation faded rapidly and was gone by the end of the first month.

One objection to the use of parenteral iron has been based on the fear of producing exogenous hemochromatosis. If one considers the amount of iron needed to produce such a disease, viz., approximately 25 gm., in comparison with a maximum 5 gm. therapeutic dose in the adult, there is a fivefold safety factor.[1] In infants, receiving between 100 and 400 mg., the safety factor probably is much higher. One would not expect this to be a problem as long as the proper dosage is used and a safe maximum is not exceeded. In our experience it has not been necessary to administer more than a total dose of 400 mg. of elemental iron. One may use a comparison of the therapeutic dose of iron-dextran complex and the amount of elemental iron in a whole blood transfusion; 250 cc. of erythrocytes contains 275 mg. of elemental iron. One usually does not think of hemochromatosis in terms of 1 pt. (about 0.5 liter) of transfused whole blood, and the amount of iron contained therein is equal to the average parenteral iron dose given in the form of iron-dextran complex.

Having proven that the parenteral therapeutic agent is efficacious, one must then decide what criteria will be used as indications for the use of a parenteral form over an equally effective oral form of the drug. In the case of iron therapy the indications for parenteral therapy are rather clear-cut; intolerance to or poor absorption of orally administered iron, gastrointestinal disease adversely affected by orally administered iron, and the failure of orally administered iron to create adequate iron stores.[1] To these indications a pediatrician may wish to add another, namely, the unreliability of some mothers. With due regard for the complex maternal-infant relationships responsible for the development of iron-deficiency anemia, one must consider that

such relationships will be effective in undermining or making impossible the successful oral therapeutic regimen in many instances. Many patients were referred for parenteral therapy for this reason.

It would seem that the creation of iron stores plus the assurance that the iron is available for rapid utilization would be strong indications for the use of parenteral iron.

It may be assumed that the use of iron-dextran complex will be abused as is that of any new drug or common medications such as antibiotics, liver, and vitamins. This should not be an argument against its use. Its utilization as a therapeutic test will circumvent the usual doubt that often arises in the physician's mind, namely, "Was the oral iron given?" Failure of response should make him suspect that the diagnosis of simple iron-deficiency anemia may be incorrect. Valuable time may be saved in this way. The replacement of the potentially hazardous blood transfusion with this safe rapidly effective form of iron will do much to help the severely anemic infants previously, and almost routinely, treated with a whole blood transfusion.

Conclusion

Intramuscularly administered iron-dextran complex, when used in the proper dosage in the treatment of bona fide cases of iron-deficiency anemia is a safe and rapidly effective method of correcting such a deficiency.

With this simple and effective method there should never be need for the potentially hazardous and relatively expensive blood transfusion in the treatment of simple, uncomplicated iron-deficiency anemia.

Summary

Sixty-five cases of iron-deficiency anemia in infants and children have been treated successfully with an intramuscular iron-dextran complex (Imferon). Correction of the anemia was rapid and without untoward effects. The indications for parenteral iron therapy are discussed.

The Children's Hospital staff pediatricians allowed the use of their patients for this study, and the resident pediatricians cooperated in collecting information and coordinating certain phases of this study.

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Trichobezoar as an Expression of Emotional Disturbance

Report of Two Cases in Children

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Trichobezoar is a relatively rare condition. About 180 cases have been reported in the medical literature over a period of 177 years.[1] We are reporting two cases which were seen on our service within the relatively short period of four weeks. We would also like to emphasize the psychological aspects of the condition with respect to etiology and comprehensive treatment.

Trichobezoars are usually found in emotionally disturbed persons.[2,3] Approximately 90% of reported cases have involved females. De Bakey and Ochsner[4] reported that 17.6% of such cases occurred in the first decade, 36.6% in the second decade, 27.4% in the third decade, and 4% in the fourth decade. Characteristically, there is a history of trichophagia, halitosis, and anorexia, especially for solids. A history of vomiting, abdominal pain, and weight loss may occasionally be elicited. Significant physical findings are limited to a palpable abdominal mass and slight to moderate wasting. Laboratory findings may show a hypochromic iron-deficiency anemia. Gastroduodenal x-ray studies employing a small amount of thin barium will usually show characteristic findings. Occasionally, a plain film of the abdomen is all that is necessary for diagnosis (as in our Case 2).

The immediate therapy is operative since ulceration, perforation with peritonitis, and obstruction are the usual complications in cases in which surgery is delayed.[5] The mortality in cases not operated on is reported as 72.7%, and the surgical mortality is less than 4%.[1] Occasionally there is recurrence, and other emotional manifestations may develop if psychiatric care is withheld.[2]

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Report of Cases

CASE 1.—A 32-month-old girl was admitted on Feb. 8, 1956, with a diagnosis of trichobezoar. The child first complained of intermittent abdominal pain five months prior to admission. This symptom persisted until the time of hospitalization. There was gradual onset of anorexia and failure to gain weight noted shortly prior to the appearance of abdominal pain. The patient had been known to eat hair from her head and from brushes ever since she was 18 months of age. Her head was shaved when she was about 26 months of age in an effort to discourage the habit.

On Jan. 27, 1956, the patient was seen privately by one of us (W. W. C.) because of the mother's apprehension following the child's first Salk vaccine inoculation. The history of trichophagia and weight loss coupled with physical findings of a palpable abdominal mass suggested the diagnosis. She was referred for x-ray of the abdomen the next day. The results are shown in Figure 1.

Psychosocial History.—The psychosocial history is significant. The mother was 17 years of age at the time of the patient's birth. She had not completed high school. The father was 18 years of age and in college, aiming for a career in engineering. Because of family responsibilities, the father had to leave college in order to accept work in a governmental agency providing $2900 per annum. The family lived with the maternal grandmother, who completely took over the care of the newborn infant and urged the mother to return to high school, which she did. The relationship between the grandmother and the mother was highly pertinent. The mother felt that she had been pushed

Fig. 1 (Case 1).—Stomach with barium added. The space-taking mass is seen in the middle third.

aside while the grandmother was again enjoying motherhood with the infant. Mother said, "I got scared to handle the baby. My husband lost confidence in himself. I felt out of place. I didn't want to touch [the baby]. I might hurt her."

When the baby was 5 months of age, the family moved into their own apartment, with the grandmother a frequent visitor. The mother felt hurt that the infant preferred her grandmother to her, but she never felt free to express any resentment toward her own mother. She seemed to project her negative feeling onto the unresponsive child. Mother said, "she would go to mother. Everything with [the baby] is a special effort. If I could just have [her] from the start again. . . . [She] would look at me with hate in her eyes, and pull out hunks (of hair) which I know would hurt her. . . . I have never seen her really laugh in my life. . . . I have bought her everything. I don't get anything out of it. She has never really stopped giving trouble. She had a good appetite though until two weeks ago when she refused to eat anything."

When the patient was about 20 months old, a sister was born. The mother states that this child was wanted all along and that she enjoyed her much more than she did the patient. She felt as though this was her first child.

Examination and Course.—Throughout the patient's hospitalization, she was solemn, withdrawn, and fretful. She was never, even on subsequent occasions, sufficiently accessible and cooperative for definitive intelligence testing, but history, language, and play ruled out mental deficiency as primary causation. The disturbance appeared to be in the mother-child relationship resulting from un-met dependency needs and a family pattern of difficulty in overt expression of hostility.

Significant physical findings on admission were limited to moderate wasting and pallor and a palpable abdominal mass.

A series of gastroduodenal x-rays revealed large trichobezoar of the stomach and hypertrophy of the pyloric muscle. Hematological findings were within normal limits. The patient was operated on on Feb. 14, 1956, with removal of a trichobezoar measuring 13×3×3 cm. (Fig. 2).

Recovery was uneventful, and the patient was on a liquid diet three days postoperatively and on regular diet within two weeks. Weight gain and appetite were satisfactory. She was discharged on Feb. 21, 1956.

The child and parent were seen by the clinical psychologist (E. F. G.) for several interviews. An attempt was made to interpret the child's behavior and her needs to the mother. It was hoped to prepare the mother to accept case-work help, but, being in the latter months of her third pregnancy, the mother did not seek this assistance. The mother felt that she had been helped by the hospital staff to meet the patient's needs more fully. She was delivered uneventfully of her third daughter. One month after the baby came home (four months after the patient's operation), there had been no recurrence of hair pulling or hair eating.

Case 2.—An 8-year-old girl was seen at Freedmen's Hospital by one of us (V. L. A.) on March 8, 1956, and was admitted with a diagnosis of trichobezoar.

Fig. 2 (Case 1).—Trichobezoar removed from stomach.

The chief complaint at the time of admission was abdominal pain and vomiting. The present illness was apparently of acute onset, beginning with nausea, vomiting, and intermittent abdominal pain on March 2, 1956. These symptoms persisted until the time of admission. On further examination of the history of the present illness, it was elicited that trichophagia was first noted at the age of 4 years and that it was not until the patient was 6 years of age that the habit was abandoned. At about the age of 6 years the patient began complaining of intermittent abdominal pain lasting 15 to 30 minutes and recurring at irregular intervals. One month prior to admission the abdominal pains became severer and more frequent. During the severe episodes, she was given sodium bicarbonate (baking soda) and put to bed. Occasionally, after a period of sleep from one-half to two hours' duration, the patient would awake free from pain and was able to take nourishment. The child had been anorexic for an indefinite period and frequently took only liquids.

The family history was negative for chronic or familial diseases. The past medical history was essentially noncontributory with respect to the chief complaint.

Psychosocial History.—The patient is the second child in a family of seven children, the youngest of whom is 4 months of age. The psychological evaluation showed a child of at least average intelligence, with verbal scale I. Q. 108, performance scale I. Q. 96, and full scale I. Q. 102 on the Wechsler Intelligence Test for Children. The patient had a great deal of jealousy toward her next youngest sibling, with whom she was forced to share her things. Her open anger at the sister has been punished by switchings and isolation, during which she sucked her thumb and pulled out and ate her hair. The patient's parents have observed that the patient is their brightest and best-behaved child. She sets high standards for herself and pulls her hair and sucks her thumb doing homework or when something will not work out right. Until the past year, when she lost her job, the mother has worked out of the home, and this child received almost no individual attention from her mother. The father, too, had been unemployed for the past six months. The family's marginal level of subsistence has thereby been worsened, and economic tensions have increased.

Physical Examination.—Pertinent physical findings on admission were as follows: The appearance was that of a well-developed but poorly nourished slightly dehydrated girl, who appeared chronically ill. She was, however, well oriented and cooperative.

The eyes were sunken but not otherwise unusual. There was a marked fetid odor to the breath. A few small discrete nontender lymph nodes of the postcervical chain were palpated. The abdomen showed a visible mass which was felt to be intra-abdominal upon palpation. The mass was palpated best in the epigastrium, but extended from the left midclavicular line in the upper left quadrant to just within the midclavicular line in the upper right quadrant. There was vague but generalized abdominal tenderness. Loud peristaltic rushes were heard on auscultation. Diagnostic gastrointestinal x-ray films are shown in Figure 3.

Fig. 3 (Case 2).—A small amount of thin barium has been introduced into the stomach through a Levin tube. This film is characteristic.

Operation.—On March 9, 1956, the patient was operated upon with use of open-drop ether anesthesia, with the removal of a trichobezoar measuring 16×6×5 cm. and weighing 373 gm. When the abdomen was opened, the entire small intestine was found to be moderately distended with gas and fluid, but thorough exploration failed to reveal mechanical blockage. Preoperative clinical evidence of bowel obstruction was postulated to be due to a hair bolus which had become detached from the primary mass, producing temporary obstruction, but which passed into the colon prior to operation.

Course and Follow-Up.—Recovery was uneventful, and the patient was taking a soft diet by March 13 and a regular diet by March 16. She was discharged on March 20, weighing 49 lb., a gain of 2 lb. over the admission weight.

When she was followed in the outpatient clinic, two months postoperatively, there had been no recurrence of the hair-plucking, which had ceased two years before the surgical removal of the hair ball. The psychological findings were interpreted

to the parents, who seemed to respond with sympathy to the child's feelings, of which they had been partly unaware. No referral for case-work help could be made, because of the distance and transportation expense to this rural family.

Comment

Cases of trichobezoar should be viewed as representing a symptom complex, the basis of which is emotional stress, usually growing out of a disturbance in mother-child relationship. This leads to trichophagia, which, in turn, causes epigastric pain, with development of an epigastric mass.

It is our feeling that surgical removal of a bezoar is symptomatic therapy and, therefore, incomplete. In our opinion, the condition is psychological in origin and therapy is only begun with the removal of the hair ball. Appropriate psychological and psychiatric therapy must begin with an adequate interpretation to the parents of the emotional causation of the physical symptom.

Summary

We have presented two cases of trichobezoar, and in so doing we have emphasized the following points:

Eating hair is a sign of emotional tension which warrants comprehensive evaluation of the child and environment. Etiological clues should be sought, and the underlying stress should be removed or ameliorated.

The importance of intermittent abdominal pain as a chief symptom of trichobezoar is stressed.

Psychological reorientation of the child and parents must often be continued after surgery.

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Pediatric Formulas for the Anesthesiologist

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It is common for a surgeon or a pediatrician to say to an anesthesiologist (sometimes in a crowded room), "We're bringing a four-year-old child up right away for emergency surgery. What do you want him to have?" It is too difficult to commit yearly dosage levels to memory, and an easily learned formula is a distinct help to the anesthesiologist and to the patient as well. Formulas need no defense in medicine or elsewhere, so long as their user remembers to consider other factors occasionally, such as (in this instance) obesity, malnutrition, head injuries, somnolence, and so on. In the majority of cases the formula (or table, if the anesthesiologist has one at hand) may be used without deviation. It must be pointed out that older rules for obtaining children's doses from adult values do not apply well to drugs used for preanesthetic medication.

It is helpful, too, to know immediately which endotracheal tube is most likely to fit a child who needs to be intubated quickly. Three systems of measuring diameters of endotracheal tubes are used here: Magill, French catheter gauge (F. C. G.), and millimeters; F. C. G. equals three times the number of millimeters. While adult drug requirements and laryngeal measurements vary little if at all, these values change from year to year in the child.

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1. Diameter of child's endotracheal tube: The Magill number (a is the child's age in years) is

$$\frac{a}{2}$$

2. Diameter of child's endotracheal tube: The F. C. G. number is

$$a+17$$

3. Length of child's endotracheal tube: The number of centimeters is

$$M+12$$

M is the Magill diameter size. Also,

$$\frac{a}{2}+12$$

$$a+10$$

$$\frac{F}{2}+3$$

$$0.8a+11$$

F is the F. C. G. number.

4. Correlation of F (F. C. G.) and M (Magill) systems.

$$F=2M+17$$

$$M=\frac{F-17}{2}$$

5. Child's dose of morphine, in grains: The reciprocal is

$$\frac{144}{a}$$

6. Child's dose of morphine, in milligrams:

$$0.42a$$

7. Child's dose of atropine, in grains: The reciprocal is

$$500-20a$$

8. Child's dose of atropine, in grains: The reciprocal is

$$\frac{850}{\sqrt{a}}$$

9. Child's dose of atropine, in milligrams:

$$\frac{a+4}{50}$$

Also

$$\frac{3}{25-a}$$

Formulas 5, 7, and 8 give values for reciprocals of doses rather than for the doses themselves. There are at least two reasons for this. First, it is more convenient to divide 144 by 8 than to divide 8 by 144, and, second, in any case, a fraction, not a decimal, is wanted, as doses are always expressed (for grains) as one over something (one eighth, or one twenty-fourth) and these are, of course, reciprocals.

I do not pretend that laryngeal diameters, tracheal lengths, and drug requirements follow these simple formulas exactly. Formulas more nearly perfect than these would lose infinitely more through awkwardness than the little that might be gained in accuracy. These formulas are easily remembered and are workable. These many formulas are offered for those who may be interested in more than every-day problems. Three or four formulas (Numbers 5, 7, and either 1 or 2) will suffice for the anesthesiologist's daily work.

When applying Formula 1 to a child whose age is an odd number of years, I have found that dropping the half-size usually works very well; thus, a No. 4 Magill tube would be used for a 9-year-old child. In any event, it is always wise to have three tubes ready, the one selected by the formula and tubes one size smaller and one size larger, but the value of the formula consists first in knowing which three tubes to prepare, and second in the statistical fact that the size obtained by the formula almost invariably works.

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Sudden Unexpected Death in Infancy

Observations at Autopsy and a Theory as to Its Mechanism

MAJOR DANIEL STOWENS (MC), U. S. Army

Sudden unexpected death in infancy, long a subject of interest, has assumed greater statistical significance with the steady decline in deaths from other recognized causes in infancy. This is attested to by the increasing frequency with which articles on the subject of sudden unexpected death are encountered in the medical literature.[1-14] Theories purporting to explain the phenomenon of sudden death are almost as numerous as the articles. In general, they may be divided into two groups: those based upon observations in a few cases and which are not applicable to a large series and those based upon minor tissue alterations commonly found at autopsy, the importance of which has not been supported by clinical observations or bacteriologic or virologic investigations. The present study was undertaken in order to evaluate the information that could be gained purely from autopsy materials.

Source of Material

At the Armed Forces Institute of Pathology cases are received from all service hospitals. The hospital at which the autopsy is performed submits duplicate slides, paraffin blocks, blocks of wet tissue, and a copy of the gross autopsy protocol. Clinical data are usually incorporated into the protocol. The pediatric section receives all cases of children less than 14 years of age, about 5000 cases per annum, approximately half of them autopsies. It is from this source that the 200 cases of sudden unexpected death were drawn over a period of two years.

Criteria of Selection

The cases utilized in the study were selected purely on the basis of history and included only those in which the infants were ostensibly well until the last moment they were seen alive. Specifically omitted from the study were those cases in which there was a history of "sniffles" or "loose bowels," for such minor symptoms are the precursors of many infectious diseases which may run a fulminant course, and the failure to recognize such a disease was not believed warrant to indict one of its possible predictable outcomes as "unexpected." Further, because of the stress created by the death of an infant, it is possible that some parents may attempt to absolve themselves of feelings of guilt by minimizing or exaggerating symptoms, the significance of which they cannot assess.

Results

Frequency.—Sudden unexpected death occurred 200 times in the series of 5000 pediatric deaths (4% of total). In the age group in which sudden unexpected death is commonest, 1 through 5 months, there were 908 autopsies. The death of 148, or 16.3%, was sudden and unexpected, making this the third commonest "cause" of death in the age group. Acute infections accounted for 44%, and congenital malformations, for 26%.

Age, Sex, and Race.—The peak incidence of sudden unexpected death in this series is in the second month of life, with the median age at 3½ months. Its frequency falls rapidly after the age of 6 months, and only 21 patients (10.5%) were over 1 year of age. Correlation of age with anatomic data revealed that sudden death in the older group usually was the result of a specific, clearly recognizable disease, whereas in the younger patients recognizable patterns of disease were absent. The age distribution of the series is shown in Figure 1.

The development and physical status of the infants were assayed by utilizing the "percentile" graphs of the Boston Chil-

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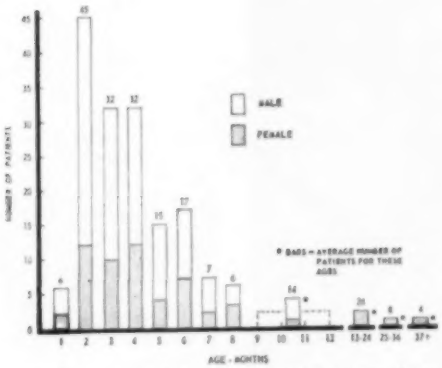

Fig. 1.—Age distribution of 200 children dying suddenly and unexpectedly. The numbers 24 and 4 over barred starred columns should be 11 and 2 respectively.

dren's Hospital. The average for the entire group was in the 57 percentile, and the range for the individuals covered the entire spread of the graphs. There were 128 boys and 72 girls, a ratio of almost 2:1. This is in conformity with the observations in all other series. The racial distribution, 160 Caucasian, 27 Negro, 7 other, and 6 unkown, is proportional to the racial distribution in the population from which the series was drawn. Only seven of these infants were prematurely born (3.5%). This is less than has been reported in other series and is not proportional to the percentage of prematurity in the total population (14%).

Diagnosis Confirmed by Pathological Examination in Thirty-Six Infants

	Cases, No.
Cerebral degeneration, cause unknown	2
Sickle-cell anemia	1
Diabetes	1
Meningitis, meningococci	5
Overwhelming sepsis	4
Congenital goiter	1
Horseshoe kidney	1
Pneumonia	3
Laryngotracheobronchitis	3
Rupture of stomach (congenital defect)	1
Congenital heart disease	1
Subdural hematoma	1
Pharyngitis	1
Giant-cell pneumonia	1
Adrenal hypoplasia	2
Cytomegalic inclusion disease	1
Tracheitis	1
Aneurysm, middle cerebral artery	1

Anatomic Observations.—The only consistent abnormality noted on gross examination was an increase in the weight of the lungs. This was of the order of 50% to 125%, with use of the weights given Coppoletta and Wolbach as standard, and was observed in 182 patients.[15] Isolated changes relative to specific diseases were also seen and will be discussed in greater detail. Gross pulmonary edema was apparent in 123 infants, and gross pulmonary hemorrhage, in 23.

Evidence of vomiting was noted in 92 patients, and mention of copious feces in

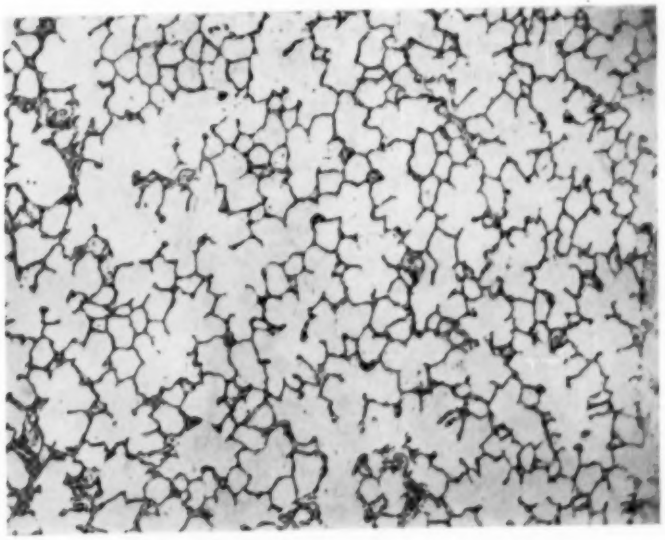

Fig. 2.—Sudden unexpected death, lung. All the alveoli are greatly expanded. There is complete absence of any sign of inflammation. (A. F. I. P. 697164.) Hematoxylin and eosin; ×46.

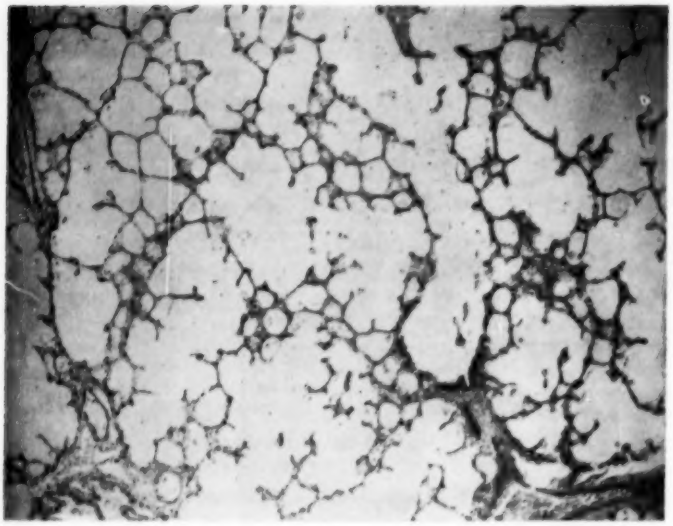

Fig. 3.—Sudden unexpected death, lung. Marked overexpansion of all alveoli. Note that the small bronchiole to the right center of the photomicrograph is also dilated and that there are no signs of infection. (A. F. I. P. 690425.) Hematoxylin and eosin; ×35.

the diaper at the time of examination was made in the autopsy protocol of 47.

Microscopic Examination.—Thirty-six (18%) of the patients manifested unequivocal signs of specific disease. Eighteen different diagnoses were involved. These are listed in the Table. In most infants (164, or 82%) no pathologically distinct entity could be recognized. In almost all of these (157 cases) the outstanding lesions were pulmonary edema and overdistention of the alveoli observed either singly or combined in various degrees. Illustrative examples are presented in Figures 2, 3, 4, and 5. Pulmonary hemorrhage was evident in 21%. In all cases at least six sections of the lungs, one of the trachea, and one of the larynx were examined, as well as at least one section from each of the viscera. Minute collections of lymphocytes in various organs, without other evidence of inflammation, were not considered proof of infection, for

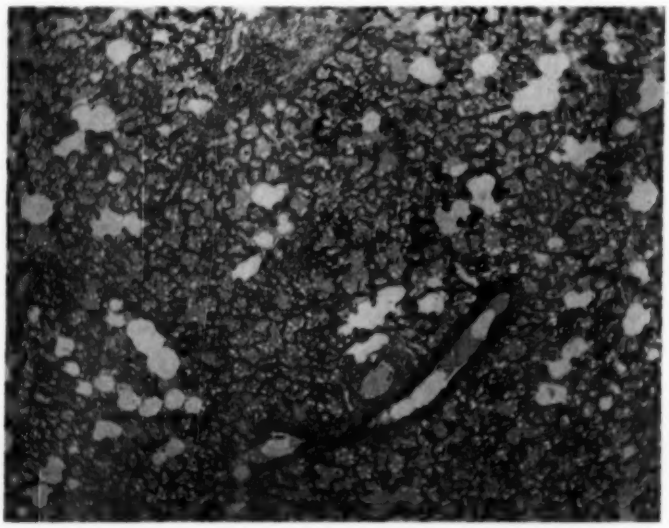

Fig. 4.—Sudden unexpected death, lung. Pulmonary edema. Note that most of the alveoli are filled with pale-staining edema fluid. Some overexpansion of the alveoli is also present. There is no inflammatory reaction. (A. F. I. P. 699651.) Hematoxylin and eosin; ×150.

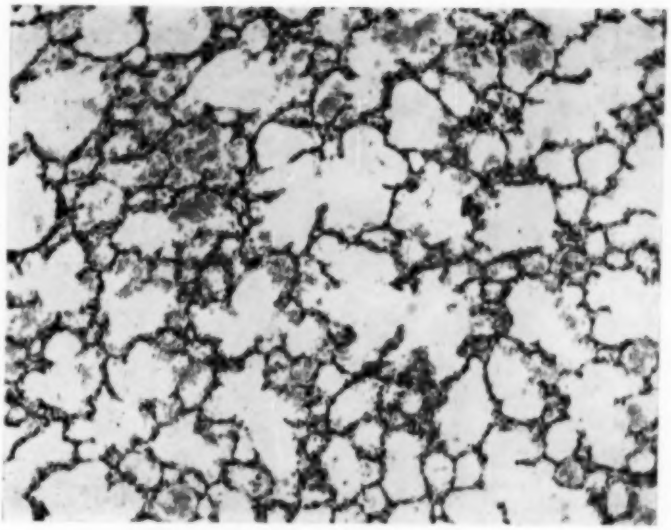

Fig. 5.—Sudden unexpected death, lung. A small amount of fresh hemorrhage is associated with generalized overexpansion of the alveoli. There is no inflammatory reaction. (A. F. I. P. 695545.) Hematoxylin and eosin; ×75.

such cellular foci can be found frequently in the tissues of persons dying of causes not related to infection.

Bacteriologic Studies.—The results of cultures of blood from the heart and of lung tissue were available in 116 cases. Thirty-one different organisms were recovered, but the only one recognized as pathogenic was the hemolytic coagulase-positive Micrococcus pyogenes var. aureus, which was identified in seven instances.

Virologic Studies.—The recovery of a virus was attempted in 41 cases. In none was a virus found.

Comment on Results

The number of cases in which a definitive diagnosis was made is smaller than in most published series. This is attributable to three factors: First, this series included only patients who were completely asymptomatic prior to death, whereas in most other series the criteria do not exclude those infants whose terminal illness appeared so mild that a fatal outcome was not anticipated or medical aid was not sought. The second factor is the availability of medical care for most service personnel. Medical officers are consulted for relatively minor reasons, and thus lesions are discovered which might otherwise be unsuspected. Finally, in this series the criteria for the histopathological diagnosis of some diseases were more stringent than in other studies.

Adrenals.—In only two cases was the weight of the adrenals more than 20% below average. These two cases are indicated in the Table. The significance of the small size of the adrenals is questionable, however, for no other evidence of adrenal insufficiency was present. Fat stains performed on the adrenals revealed no evident lack of lipid. In no instance was hyperplasia of the adrenal encountered.

Thymus.—The weights of the thymuses were found to be more variable than those of any other of the viscera; thus, the significance of the striking enlargement that was seen in several cases cannot be assayed. However, in the few cases in which the thymus was very large there was no evidence of tracheal compression. The average weight of the thymuses for the group was twice that of a control series.

Consideration of Other Factors

Home Investigation.—In 57 instances the circumstances surrounding the death of the infant were investigated by medical and police authorities. In none was evidence of foul play or parental neglect discovered.

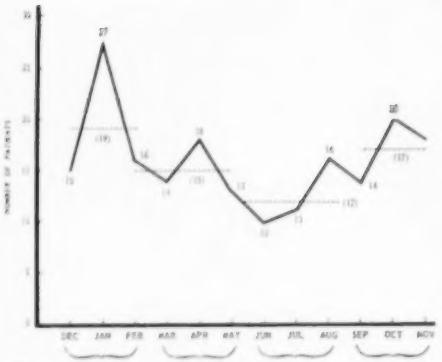

Fig. 6.—Seasonal distribution of sudden unexpected deaths in 200 children.

History of Contact with Infectious Diseases.—In 47 cases an attempt was made to gather this information. In 13 a history of respiratory illness of a minor nature in a sibling was elicited.

Seasonal Incidence.—This information is presented in Figure 6. A definite peak is reached in January, but deaths occurred at all times. It must be remembered, however, that this material is drawn from a wide geographic distribution and, therefore, the significance of these data is equivocal.

Control Series.—For purposes of comparison two control series of cases were studied. The first consisted of 50 cases of pneumonia which were recognized clinically and proved bacteriologically or virologically. Many of these ran courses of less than 24 hours. In all cases an inflammatory reaction in the lung could easily be recognized, and in many there was also reactive hyperplasia of the lymphatic tissue throughout the rest of the body. The second series consisted of 35 cases of pathologically proved interstitial pneumonia.[16] Although the course of the disease was short in some of these children, in most it was longer than that of bronchopneumonia. Evaluation of the histories in these cases revealed that, even though at times the symptoms were not alarming, there always were clinical symptoms, either specifically of a respiratory infection or of a more generalized nature, such as vomiting, fever, or restlessness.

In regard to the problem of interstitial pneumonia, the great elasticity of the infant's lung tissue must not be overlooked. It is because of this feature that microscopic sections of the lung often appear collapsed and the outlines of the alveoli and alveolar walls are indistinct and thickened. This somewhat resembles the picture of interstitial pneumonia, but it can be distinguished from that condition by the absence of inflammation.*

Comment

The primary purpose of this exercise was to determine what could be learned of the nature and cause of sudden death from autopsy studies. It seems apparent from the results that the anatomic changes noted do not conform to the pattern of any distinct, pathologically recognizable, disease. It is believed, however, that the tissue alterations which are found, in conjunction with the negative results of the bacteriologic and viral studies, are sufficiently consistent to establish this syndrome as a distinct entity. In a negative sense a considerable amount was accomplished. The absence of the usual signs of infectious disease in most of the cases was striking. It is true that minute collections of inflammatory cells were seen in various parts of the respiratory tract, but, in my experience, similar collections can be found in many children dying suddenly from any cause, and their signficance is believed to be minimal and quite disproportionate with the reactions to infection seen generally in children. For example, in the sections of lung taken from a child dying of bronchopneumonia it is often difficult to find a single alveolus free of exudate (Fig. 7). The problem as far as pneumonia is

* The hazard presented by artifact in these cases can be avoided by instillation of fixative under moderate pressure (40 to 50 mm. H_2O) into the tracheobronchial tree at the time of autopsy and fixation of the lung *in toto*. Such a procedure partially expands the lung and allows the alveolar walls to be seen clearly in microscopic section.

Fig. 7.—Bronchopneumonia. This 3-month-old child died on the third day of illness. There is complete absence of any apparently functional alveoli. This field is representative of the entire lung. (A. F. I. P. 697762.) Hematoxylin and eosin; ×85.

concerned is more often "How did the child manage to survive as long as he did?" rather than "Why were such minor lesions lethal?" Add to this the consistently negative or inconclusive results of bacteriologic and virologic studies in this and other reported series and insistence upon the infectious nature of this syndrome becomes difficult to defend.

The consistency with which pulmonary edema and emphysema of varying degree were noted was the single most striking feature of the series as a whole. Although neither of these processes appears to be of lethal magnitude, they are found so constantly that it seems logical to relate them to the fatal pathophysiologic catastrophe.

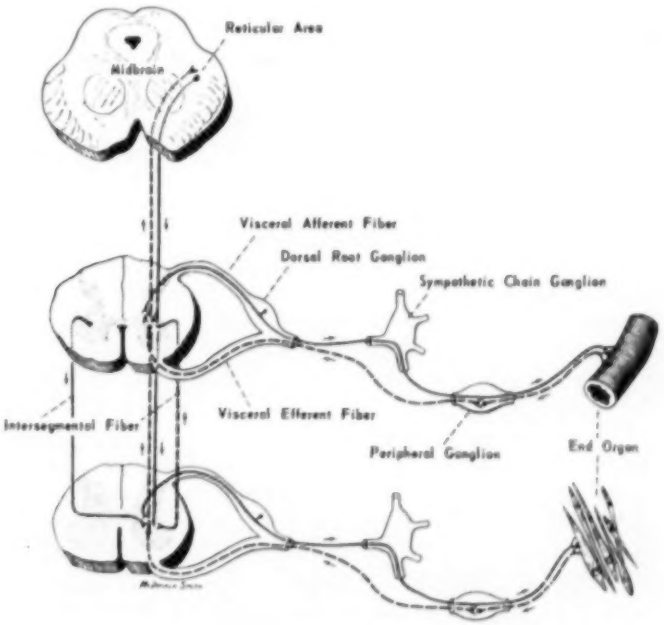

Fig. 8.—A diagrammatic representation of the pathways conveying the viscerovisceral reflexes.

Briefly, the causes of emphysema are (*a*) forceful overexpansion of the lungs, (*b*) interference with expiration, and (*c*) loss of elasticity of the alveolar walls. The first and last of these do not appear applicable in this instance. Interference with expiration may be accomplished by a variety of means, for instance, obstruction to the air passages by foreign material or tumor, laryngospasm (as seen in drowning), external compression, and bronchospasm. Bronchospasm appears the most likely mechanism to invoke in this problem, for evidence of the other means would, it is believed, be visible were they present. Whatever the cause of bronchospasm, the initiating impulse is transmitted through the autonomic nervous system, in the fibers of the vagus. The initial stimulus may be of a reflex nature, as seen in bronchospasm during abdominal surgery.[17] The pathways involved have been studied extensively and lie entirely within the autonomic nervous system. These are the viscerovisceral (or vagovagal) reflexes.[18-20] The pathways involved are illustrated in Figure 8.

The actual mechanism involved in the production of acute pulmonary edema is not known. For the purpose of this study, however, it is believed that what is significant is the reflex nature of pulmonary edema, as shown by its occurrence in lesions of the central nervous system,[21] especially in children.

Two other phenomena of a reflex nature were also noted in these children. Mention is made of copious amounts of feces in the diaper in 47 patients and evidence of vomiting in 97. It is believed, on the basis of these observations, that the mechanism of death in these infants is related to a mass viscerovisceral reflex.

Of greatest significance is the fact that this syndrome is one of young infants; median age, 3½ months. Its frequency falls rapidly after 6 months of age, and it is rare in older children and adults. The 3-month-old infant is physiologically immature in many ways, but anatomically his lack of development is nowhere so striking as in the cerebral cortex. This is of interest for the following reason: The impulses of the autonomic nervous system are mediated through the midbrain. In the reticular area of the midbrain such reflexes are potentiated. Experimentally, it has been shown that responses to subminimal stimuli can be obtained when there is simultaneous stimulation of the reticular area.[22] It is also known that the cerebral cortex is inhibitory to the lower centers. It is possible, therefore, that in the immature central nervous system the reticular area is relatively more active because of the absence of inhibitory impulses from the partially developed cerebral cortex. Because of this relatively greater potentiating effect, the viscerovisceral reflexes in infants are of greater magnitude and consequence than in older children and adults.

The stimuli initiating such reflexes may be numerous. Probably oftenest they are minor foci of infection, usually in the respiratory tract, for these are very frequent in themselves. It is possible that external physical and even sensory stimuli may be sufficient to excite a response.

The pulmonary effects of the reflex are probably not lethal. It is only because of the structure of the pulmonary tissue that it produces any visible effects. The reflex is a mass one, affecting all viscera, and most probably it is through the effect on the heart —either by arrest or by initiation of abnormal rhythm—that it is lethal.

That the autonomic nervous system of infants sometimes functions imperfectly is manifest in its grossest fashion by the occurrence of a clinically recognized and recognizable disease—familial dysautonomia.[23] Whether milder forms of this disease exist is not known.

It may be argued that such a hypothesis can neither be proved nor disproved and that it is valueless because it fails to provide any prognostic criteria. This is true at present, but it is hoped that clinical investigation of autonomic function in infancy may uncover a means of detecting potential or partial

autonomic imbalance. The hypothesis is also believed to be of value if it provides a possible explanation for this phenomenon other than one dependent upon minimal or nonexistent evidence, either clinical, pathologic, bacteriologic, or virologic.

Finally, it is one of the purposes of this work to demonstrate that sudden unexpected death in infancy exists as a distinct entity, not related to infectious diseases, congenital malformations, or as yet known chemical or physiologic malfunctions. It is believed that progress toward the solution of the problem can be assisted by honest admission of our ignorance but will be hindered by continued insistence upon fitting this phenomenon into one of the recognized categories of disease.

Summary

Two hundred instances of sudden unexpected death in infants and young children were studied. Only the lungs showed any alteration from the normal, the changes consisting of generalized overexpansion of the alveoli and pulmonary edema. No inflammation was found. Evidence is presented to demonstrate that death in these patients was not the result of infectious disease. The hypothesis is advanced that death in these patients is the result of a generalized neural spasm mediated through the autonomic nervous system.

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Thyroid Dysfunction in Goitrous Children

Radioiodine Studies and Plasma Chromatograph Analysis for Thyroid Hormone

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In 1943, Hamilton and his associates [1] recognized that the measurement of iodine uptake by the thyroid may not indicate the degree of thyroid function measured in terms of thyroid hormone activity. They reported two goitrous children, both of whom demonstrated clinical and laboratory evidence of marked hypothyroidism, and yet both accumulated more than normal amounts of iodine following tracer doses of I^{131}. At the time, because of technical difficulties, they were unable to measure circulating thyroxin. Biopsy specimens were taken from the thyroids of these patients, and radioautographs showed that in both most of the iodine was deposited in the cells of the acini rather than in the colloid and surprisingly large amounts of iodine were concentrated in the thyroid. They suggested that, "if the circulating thyroxine is extremely low or even absent in such patients, the metabolic fault must lie in the inability of the thyroid to release, rather than in its inability to produce, this hormone." Their conclusions paved the way for the many subsequent investigations dealing with normal and pathological chemistry and physiology of the thyroid gland.

In 1954, Wilkins et al.[2] studied four patients with congenital hypothyroidism who had thyroids which readily accumulated and retained I^{131}. The thyroids were not enlarged in two of the patients, but the other two developed goiters when they were 6 and 8½ years old, respectively. Protein-bound iodine of the plasma and I^{131} studies were not done on these patients. Wilkins postulated that there must be some defect in the ability of the thyroid to produce thyroid hormone in their patients.

During the four years prior to Wilkin's report, Stanbury,[3] Hubble,[4] and McGirr and Hutchison [5] had demonstrated the accumulation of I^{131} in the thyroid of nonendemic cretins and failure of release of hormone by the thyroid gland. Stanbury [6] was the first to identify precursors of thyroid hormone in the blood and substantiate the hypothesis of defective hormone synthesis in certain cases of nonendemic goitrous cretins. These, he classifies as inborn errors of metabolism.[7]

It is believed that enlargement of the thyroid gland of persons with congenital nonendemic goiter is a compensatory mechanism in response to defective synthesis of thyroid hormone.[6]

Three different defects have been recognized. The defect in one group is that the thyroid is incapable of incorporating accumulated iodide into organic combination.[5, 8,9] I^{131} accumulates rapidly in the gland after administration, but it is discharged rapidly from the gland after the oral administration of potassium thiocyanate. The thiocyanate ion displaces iodide ion but not organically bound iodine from the gland. The defect in a second group is that the thyroid can bind accumulated iodide into mono- and diiodotyrosine, but then only small amounts of thyroxine are formed.[6,9] The accumulated labeled iodine is not released rapidly from the gland, and no labeled substance other than iodide and thyroxin is present in the blood. It has been suggested that the metabolic defect in the second group is at the step in hormone

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synthesis where iodotyrosine groups are coupled to form the complete thyroid hormone.[9] The defect in a third group is that the thyroid cannot deiodinate the hormone precursors and they are mobilized into the circulation.

In two of Hubble's[4] cretinous siblings with goiter, I^{131} accumulated rapidly and to an abnormally high level in the thyroid. Chromatographic methods revealed thyroxin in one of the glands. Potassium thiocyanate failed to discharge appreciable amounts of I^{131} from the thyroid of the other. McGirr and Hutchison[5] found abnormally high levels of protein-bound I^{131} in the sera of several similar patients. Chromatographic analysis of the serum of one patient revealed the presence of an iodinated substance which was not thyroxin. They inferred that this might be diiodotyrosine, but the substance was not identified. Several of the patients of Hubble[4] and McGirr and Hutchinson[5] and one of Stanbury[6] showed the rapid release of the labeled iodine from the gland after peak uptake was reached.

Mono- and diiodotyrosine occur normally in the thyroid but do not occur normally in the blood. Prior to the recognition of these compounds in the blood of goitrous cretins they had been demonstrated in the blood only after heavy irradiation to the thyroid gland. Mono- and diiodotyrosine are mobilprocess of hormone secretion is initiated by TSH stimulation. However, the hormone ized along with thyroid hormone when the precursors are deiodinated and degradaded before they can pass through the thyroid-blood barrier. The case of Stanbury[6] demonstrated a defect in the regulation of the precursors, because they were released into the blood.

A recent study by Stanbury's group[11] demonstrated that certain persons with familial goiter are unable to deiodinate diiodotyrosine when this substance is injected intravenously. Tissue slice studies revealed that I^{131}-labeled diiodotyrosine was deshalogenated by thyroid tissue from cows, sheep, five patients with nodular goiter, and one patient with treated hyperthyroidism. However, thyroid tissue slices from a patient with familial goiter and cretinism failed to deshalogenate labeled diiodotyrosine. They conclude that "the metabolic disorder of the patient with familial cretinism and that of similar patients is conceived as a general somatic failure of the normal process of deshalogenation of mono- and diiodotyrosine. The hypothyroid state and the hyperplastic goiter are presumed to result from the loss of hormone precursors from the thyroid and from the body."

Recently, we have had the opportunity to observe and study several infants and children with goiter and suggestive hypothyroidism due to inborn errors of metabolism. Three of these cases form the basis for this presentation.

Report of Cases

CASE 1.—A 6-year-old white boy is the younger brother of Patient 2. Both he and his brother were admitted to the hospital on March 1, 1956, with similar complaints.

He was the product of an uneventful pregnancy and delivery, and his birth weight was 6 lb. 14 oz. His first year was marked by eczematous skin reactions during the first six months and several upper respiratory infections during the last six months. His first tooth did not erupt until he was one year old. A roentgenogram of the wrists at age 14 months revealed that no carpal centers had ossified. Dessicated thyroid 1½ grains (97.5 mg.) twice daily was prescribed but was administered only a few weeks. He sat at 7 months, stood at 15 months, and walked and said a few words at 17½ months. Also, at age 17½ months his thyroid was first observed to be enlarged. During his second, third, and fourth years he had numerous episodes of tonsillitis, otitis media, and allergic rhinitis. At age 3 he received radiation therapy for hypertrophied tonsils and adenoids. The thyroid enlargement remained about the same until the age of 5, when there was a noticeable increase in size. At this time his serum protein-bound iodine (PBI), determined in another laboratory, was 2.9γ per 100 cc. Desiccated thyroid, ¼ grain (16.25 mg.) daily, was administered during the following three months. There was no apparent decrease in size of the thyroid.

At the time of admission to the hospital, his mother stated that she believed that he had not been growing normally. His appetite had always been poor, and his skin was dry and his hair coarse and dry. She noticed that for several months he

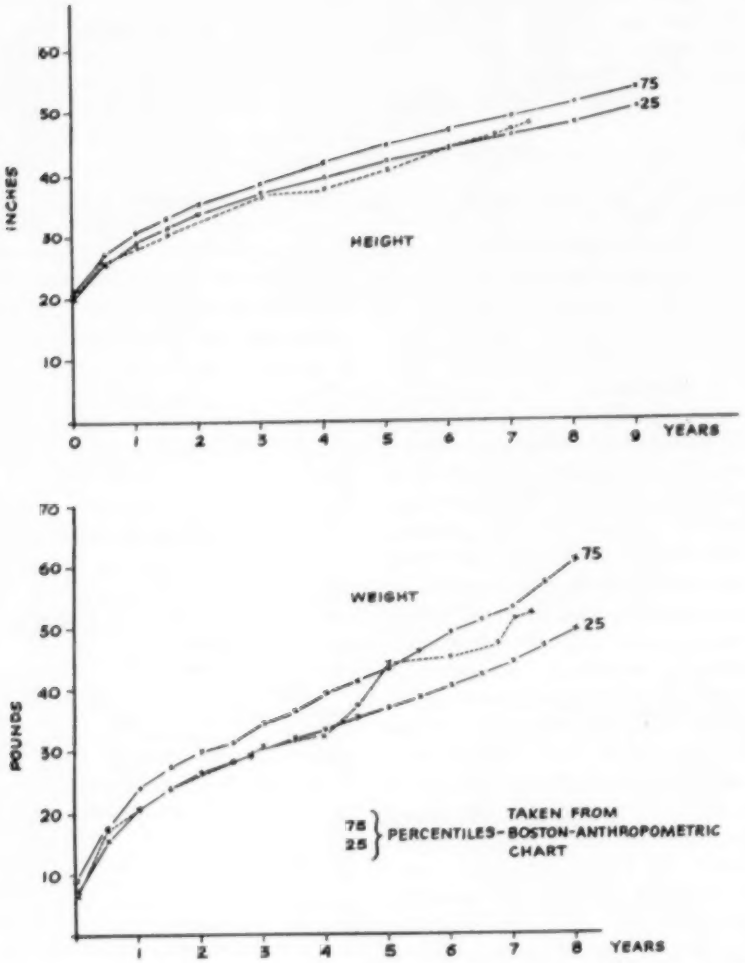

Fig. 1 (Case 1).—Height and weight curves of patient (dotted line) born Dec. 23, 1949.

had become fatigued easily. His growth curve is shown in Figure 1.

Physical examination revealed a well-developed boy who appeared short and stocky for his age. He was alert and seemed to be of average intelligence. Height was 44½ in.; weight, 47 lb.; pulse rate, 104, and blood pressure, 96/60. His hair was dry, and there was patchy alopecia. The nasal mucosa was edematous and pale. Both tympanic membranes were dull and thickened. Tonsils were hypertrophied. Both lobes of the thyroid were moderately enlarged, firm, and smooth. No nodules were palpated. The circumference of the neck was 28.5 cm. His fingers appeared shorter and thicker than normal. There were no other abnormalities.

A roentgenogram of his wrist showed only three carpal centers. The blood cell count and urinalysis showed no abnormal findings. The serum cholesterol was 123 mg. per 100 cc., with 73% esterified. Serum PBI was 6.1γ per 100 cc. Results of the radioactive iodine studies are shown in Figures 2 and 3.

Case 2.—A 9-year-old white boy was admitted to the hospital with his younger brother on March 1, 1956. The reasons for admission were goiter and slow growth.

His birth weight was 6 lb. 13½ oz., after an uneventful pregnancy and delivery. During his first six months he vomited frequently and was treated for pylorospasm. Between 18 months and 4½ years he had frequent upper respiratory infec-

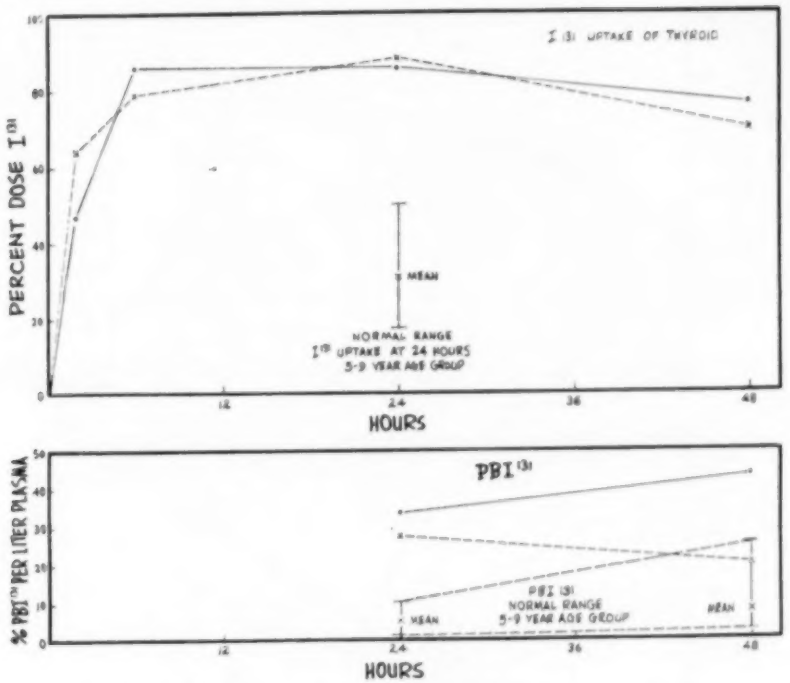

Fig. 2.—Radioiodine studies. Case 1, dotted line; Case 2, solid line.

tions and allergic rhinitis. At 4¼ years he received radiation therapy for hypertrophied tonsils and adenoids.

His development has apparently been normal, although, at the time of admission, his mother complained that he was not growing normally. He sat at 8 months, stood at 1 year, and walked at 15 months. He spoke his first words at 15 months. His first tooth erupted at one year. His growth from birth is shown in Figure 4. At present he is in the third grade and is considered intelligent.

At the age of 7 years his thyroid was first observed to be enlarged. At 8 years the serum PBI, determined at another hospital, was 3.3γ per 100 cc. Desiccated thyroid gland was prescribed, starting with ¼ grain and increased to 1 grain (65 mg.) daily over a period of one month. Therapy was discontinued after four months. During this period his thyroid decreased slightly in size. For the six months before admission he had received no treatment and the thyroid appeared to have increased in size. His mother complained that his skin was dry and his hair coarse and dry. Three weeks before admission to the hospital he had mumps meningoencephalitis, from which he recovered with no sequelae other than irritability, a change from his previously placid personality.

Physical examination on admission revealed a well-developed slender boy who was alert and intelligent. Height was 52¼ in.; weight, 63½ lb.; pulse rate, 88, and blood pressure, 110/60. His hair was dry, and the nasal mucosa was edematous

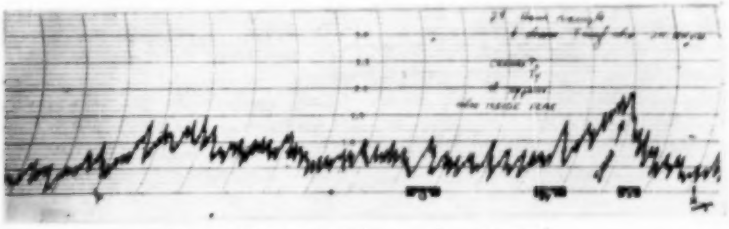

Fig. 3 (Case 1).—Plasma chromatograph.

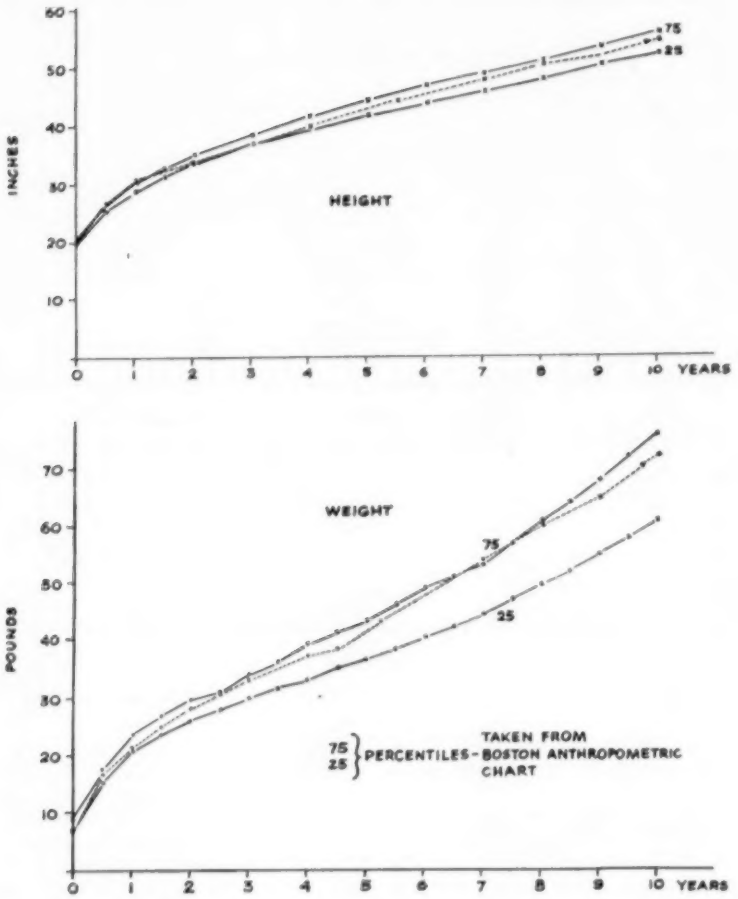

Fig. 4 (Case 2).—Height and weight curves of patient (dotted line) born March 27, 1947.

and pale. Both lobes of the thyroid were considerably enlarged, firm, and smooth. The circumference of his neck was 31 cm. No nodules were palpated. All other physical findings were normal.

Roentgenogram of his wrist showed only three carpal centers. Blood count and urinalysis revealed no abnormal findings. The serum cholesterol was 96 mg. per 100 cc., with 70% esterified. An oral glucose-tolerance test showed a fasting sugar of 72 mg. per 100 cc., 60 mg. per 100 cc. at one-half hour, 60 mg. per 100 cc. at one hour, 64 mg. per 100 cc. at two hours, and 57 mg. per 100 cc. at three hours. His serum PBI was 9.5γ per 100 cc. Results of radioactive iodine studies are shown in Figure 2.

Clinical Course.—It was concluded that the goiters of these two brothers were due to excessive thyrotropic activity due to deficient circulating thyroid hormone. For this reason, thyroxin therapy was prescribed for both. The dose of thyroxin was 0.4 mg. daily for one week and then 0.4 mg. twice a day for three months. After six weeks of therapy the goiters of both boys had almost disappeared, and after two months both thyroids were of normal size. After three months the thyroxin dosage was reduced to 0.4 mg. once a day because of nervousness, irritability, and slight tachycardia. They are being maintained on this dose at the present time.

CASE 3.—An 8-year-old white boy was admitted to the hospital on Dec. 2, 1956, for evaluation of thyroid enlargement. The neck swelling was observed by his mother for the first time about three weeks prior to admission.

His birth weight was 4 lb. 14 oz., and he was the smaller of twin boys born at term. His twin

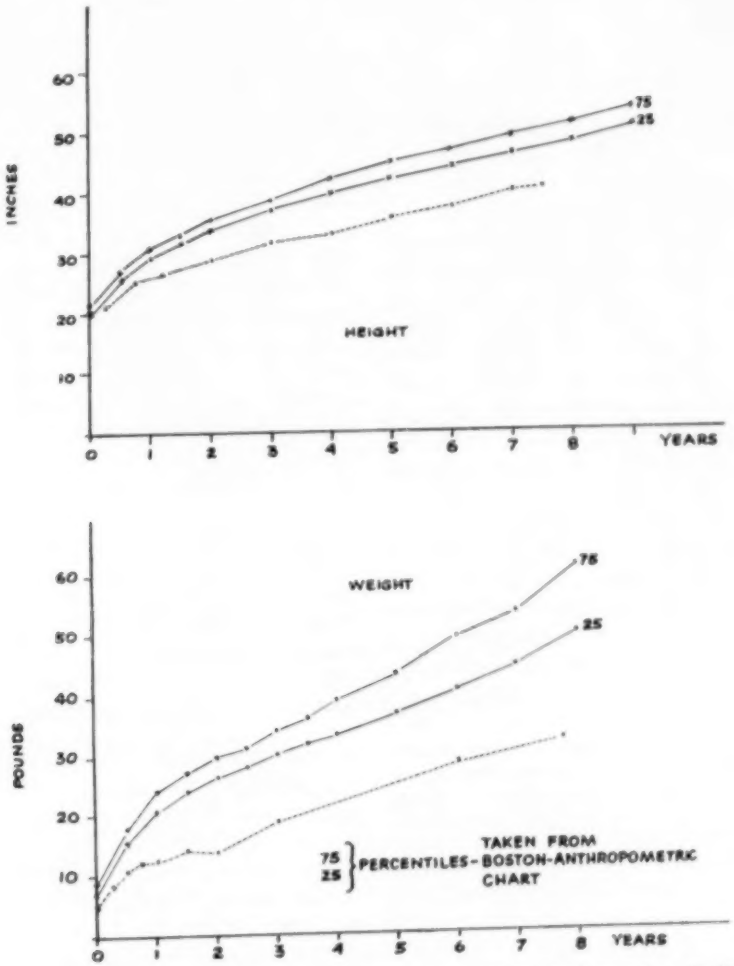

Fig. 5 (Case 3).—Height and weight curves of patient (dotted line) born Jan. 15, 1949.

weighed 7 lb. 8 oz. Delivery was uneventful. During the first two months of life he manifested spasticity and frequent vomiting and he was described as a difficult feeder. He had a large umbilical hernia, bilateral cryptorchidism, and bilateral inguinal hernias. He was circumcized at 7 months. At 3 years of age an incarcerated right inguinal hernia was repaired. His first four years were marked by frequent upper respiratory infections. He wears corrective lenses for severe myopia and astigmatism. During the year before the present hospital admission he had numerous attacks of otitis media which resulted in bilateral hearing loss, for which he now uses a hearing aid.

His growth has been consistently retarded since birth, as is demonstrated in Figure 5. During most of his infancy, he was considered to be spastic and mentally retarded. The apparent spasticity disappeared during the third year, and his mental and motor development advanced remarkably about the same time. He began to walk and talk at 2½ years. He now attends public school (first grade) and is progressing satisfactorily despite his visual and hearing handicaps.

Physical examination on admission revealed a poorly developed, only fairly nourished, alert child who did not appear acutely ill. Weight was 32 lb., and height was 43 in. Temperature was 99.8 F; pulse rate, 120; respiratory rate, 28, and blood pressure, 110/60. He was noted to have a peculiar bird-like facies, with large palpebral fissures and injected conjunctivae. He had many carious teeth.

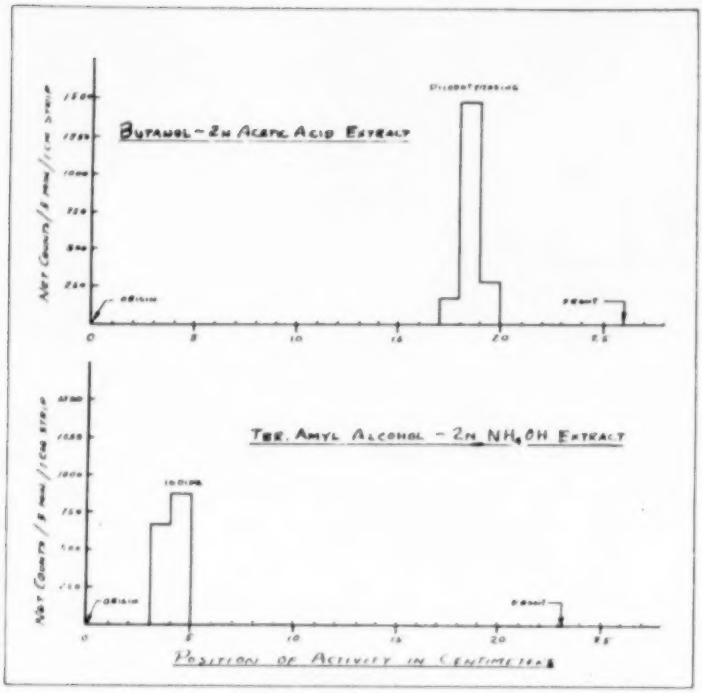

Fig. 6 (Case 3).—Chromatographic separation of 24-hour plasma sample.

Examination of his thyroid revealed general moderate enlargement, with the right lobe slightly larger than the left and a hard pecan-sized nodule in the left lobe. There was bilateral cryptorchidism, an underdeveloped slightly bifid scrotum, and a pea-sized fixed gonad in the left inguinal canal.

Abnormal laboratory findings were limited to the radioactive iodine studies. Twenty-four-hour thyroid uptake was 45%. PBI^{131} (per cent of dose per liter of plasma) at 24 hours was 0.460%. Serum PBI was 8.5γ per 100 cc. The latter two values are elevated. Chromatography of the serum at 24 hours revealed diiodotyrosine and iodide only (Fig. 6). Some thyroxin was present when chromatography was repeated at 48 hours.

Biopsy of the thyroid nodule revealed benign adenoma.

This patient has been started on desiccated thyroid therapy, but it is too early for an evaluation of effects.

Comment

The three cases all had goiter as a common feature. Prior to our first examination of the two brothers there were indications in their histories that thyroid disturbance was considered in both by their attending pediatricians. The younger boy (Case 1) was given desiccated thyroid at 14 months of age, when x-ray examination of the wrists revealed no carpal centers. The x-ray study was done because the pediatrician believed that his development was not proceeding rapidly enough. The thyroid therapy was given for only a few weeks. At 17½ months his thyroid was observed to be enlarged, and it has remained enlarged until the present (age of 6 years). Despite suspected hypothyroidism, this child's physical and mental development have been good. His growth charts (Fig. 1) reveal that his growth rate approximated the average throughout his life, although his mother was not satisfied with his progress. No thyroid or other disturbance was suspected in the older brother (Case 2) until the age of 7 years, when thyroid enlargement was first observed. His satisfactory physical development is shown

up to the present (age of 9 years) in Figure 4.

I^{131} studies were done on the two brothers because of goiters and suspected hypothyroidism. These studies are summarized in Figure 2, which shows marked increase in the uptake of radioactive iodine and significant elevations in the PBI^{131}. The serum PBI of Case 1 was 6.1γ per 100 cc., and that of Case 2 was 9.5γ per 100 cc. Since these children were not clinically hyperthyroid, it was suspected that an unusual thyroid dysfunction was present. Plasma chromatography studies * were done in Case 1 and were interpreted as showing an iodinated tyrosine compound just before the iodide peak (Fig. 3). The presence of triiodothyronine (T3) and thyroxin (T4) was also demonstrated.

The radioactive iodine studies indicate a state of thyroid gland hyperactivity. However, these findings are not consistent with the clinical picture. The only sign of hyperactivity is goiter. Paradoxically, the clinical impression has been one of border-line hypothyroidism, and x-ray studies have revealed markedly retarded bone age. The chromatography studies throw light on this discrepancy by demonstrating the presence in the blood of an iodinated nonhormonal compound in addition to thyroxin.

The third child (Case 3) was admitted to the hospital because of enlarged thyroid and associated nodule. He was obviously dwarfed and had other physical defects, but he was not considered clinically hypothyroid. His I^{131} uptake was normal, and his PBI^{131} and serum PBI were elevated. As in the case of the brothers, these findings indicated thyroid gland hyperactivity which was inconsistent with the clinical picture. In this case, also, chromatographic analysis of the serum revealed the abnormal presence of thyroid hormone precursors. The thyroid nodule was found to be a benign adenoma, histologically similar to those described by Hayles et al.[12] from cases of goitrous cretins.

* Plasma chromatography studies were performed and interpreted by Dr. Edwin Albright, of the University of Wisconsin, in Case 1 (younger child).

Conclusions and Summary

The discrepancy between clinical findings and I^{131} uptake, PBI^{131}, and serum PBI are well shown in these three cases. In two, the clinical picture was that of goiter and suggestive hypothyroidism, and the third showed goiter, dwarfism, and several congenital defects. However, the laboratory results indicated thyroid hyperactivity in the three patients. It was only after plasma chromatographic studies that the nature of the defect was revealed, viz., that of apparent failure of the thyroid to deshalogenate the hormone precursors, which were released into the plasma. Normally, the precursors are not found in the plasma. In spite of the fact that thyroid hormone was found in the blood of these patients, it is logical to assume that it was present in insufficient amounts, since peripheral signs of hypothyroidism were present. Quantitative thyroxin determinations were not done. The high PBI^{131} and serum PBI can be explained on the basis of circulating plasma iodinated hormonal precursors in addition to triiodothyronine and thyroxin.

The suggestion of clinical hypothyroidism in the two brothers and the goiters of all three patients can be explained on the basis of probable insufficient circulating thyroid hormone, resulting in increased release of thyrotropin. At present we are studying thyrotropin production in normal children and those with thyroid disease.

From the study of these three patients, it is evident that persons with inborn errors of thyroid metabolism may or may not manifest the usual clinical features of hypothyroidism. Determination of serum protein-bound iodine, radioactive iodine uptake, and protein-bound I^{131} may be insufficient to clearly define the metabolic abnormality. Chromatographic analysis of circulating radioactive protein-bound iodine compounds is necessary to define the defect. It would be helpful to know the plasma thyroxin level

in both normal children and children with thyroid dysfunction.

Cases 1 and 2 were referred by Drs. Harold Gerber and Albert F. Stein.

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Case Reports

Cushing's Syndrome in Infancy

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Cushing's syndrome in infancy is very rare. Recently, Guin and Gilbert reviewed 28 cases of adrenal cortical carcinoma associated with this syndrome in persons less than 14 years old.[1] They added one case, with onset at 6 weeks. In only 2 of the 29 cases did the symptoms appear before the age of 6 months. In reviewing the literature the impression is gained that Cushing's syndrome in the pediatric age group is usually caused by an adrenal tumor. Albright and Forbes were of the opinion that all such cases in infancy are caused by malignant tumors.[2] Forsham and Thorn also believed that the adrenal lesion was oftenest a malignant one when the disease appeared before puberty.[3] However, Powell cites two instances due to adrenal cortical adenoma within the first year of life.[4,5] Furthermore, Talbot et al. claim that bilateral adrenal hyperplasia is more commonly the cause of the syndrome than adrenal carcinoma.[6]

Several reviews of the literature were gleaned,[1,4,7,9] and a clearer view was obtained of the ratio of tumor to hyperplasia in the genesis of Cushing's syndrome in children. Chute et al.[9] critically evaluated 33 cases of Cushing's syndrome or basophilism reported in children up to the year 1949. Eighteen of these came to postmortem examination. Carcinoma of the adrenal cortex was found in eight; hyperplasia, in five; normal adrenals, in two; hypoplasia, in one, and in two the condition of the adrenals was not mentioned. It is of interest to note that the autopsy studies revealed a total of seven cases with pituitary adenoma, all occurring in the cases in which adrenal carcinoma was not present.

The purpose of this paper is to present a case of Cushing's syndrome in a 5-month-old infant caused by an apparently benign adrenal cortical adenoma. The disorder, it appeared, started within a few weeks after birth and possibly before birth.* The signs and symptoms were sufficiently well developed by the fifth month to warrant surgical interference. This case is among the youngest on record.

Report of Case

A 5-month-old white girl was admitted to the University Hospital on Feb. 18, 1957. Several weeks before admission, while she was under treatment for viral pneumonitis and thrush, her pediatrician noted marked obesity, slight enlargement of the clitoris, and the beginning of pubic hair growth (Fig. 1).

The patient was a full-term infant with a birth weight of 8 lb. 8 oz. (3855 gm.). She was breast-fed for the first four weeks, at which time she was hospitalized for five days for the treatment of pneumonitis. After this, she was started on various formula feedings, with supplemental vitamins daily. Family history was noncontributory. Four older siblings, living and well, have apparently normal growth and development.

On physical examination, the obesity was generalized, there was mooning of the face, and a fine acneform eruption was seen over the malar eminences. A small amount of pubic hair was present, and slight enlargement of the clitoris was noted. The abdomen was extremely obese,

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From the Department of Endocrinology, Medical College of Georgia. Visiting Research Fellow from the University of Mexico (Dr. Manautou).

* Since calcification was present in the adrenal adenoma, it is quite possible that the process had gone on for more than five months.

Fig. 1.—Five-month-old girl. Note moon face, obesity, mild acneform eruption on face. Slight hair growth on pubis and vulva not visible in photograph.

and the liver was palpable 3 cm. below the right costal margin. Deep tendon reflexes were generally hypoactive. Polyphagia, polydipsia, and polyuria were prominent features at this time and had been present for several months. The blood pressure was 70/30 mm. Hg, and the weight was 20 lb. 12 oz. (9410 gm.).

X-ray examination revealed normal texture of the bones of the skull and wrist, and the bone age was within normal limits. The sella was of normal size and shape; the heart and lungs appeared normal. X-ray of the abdomen demonstrated a faint shell-like calcification 2×1 cm. in size above the right kidney. This was seen on repeated films. Intravenous pyelogram indicated good bilateral kidney function. The right kidney, however, appeared depressed, with slight clubbing of its calyces. Minimal osteoporosis of the vertebrae was noted.

Laboratory data was as follows: hemoglobin 14.8 gm.; hematocrit value 47%; leukocytes 13,200 per cubic millimeter, with a differential count of 57% polymorphonuclear cells, 39% lymphocytes, and 4% monocytes. The fasting eosinophil count was 0 per cubic millimeter on two occasions. The urine was yellow, slightly cloudy, and alkaline, with specific gravity of 1.013, and it was negative for sugar and albumin. Urinary excretion of 17-ketosteroids was 6.5 mg. per 24 hours; 17-hydroxycorticoids, 9.4 mg. per 24 hours. Measurable amounts of pregnanediol and pregnanetriol were not present. The qualitative Allen test for dehydroisoandrosterone was negative. Serum sodium was 150 mEq. per liter; chloride, 101 mEq. per liter. The fasting blood sugar was 105 mg. per 100 cc.

On the third hospital day 20 units of repository corticotropin injection (corticotropin gel) was given intramuscularly and the 24-hour urine assay was repeated. There was only a slight increase in the 17-hydroxycorticoids, to 11.5 mg., with a slight decrease in 17-ketosteroids, to 6.1 mg. (Table).

Before an exploratory laparotomy could be performed, the patient became ill with an upper respiratory infection, diarrhea, and the recurrence of oral thrush. Intensive antibiotic and antidiarrheal therapy, in addition to supportive measures, was employed successfully, and the laparotomy was performed on March 9, 1957.

In addition to the usual preoperative measures, the infant was given 50 mg. of cortisone acetate intramuscularly on the eve of surgery and 50 mg. of hydrocortisone intravenously in 250 cc.

Summary of Therapy and Hormonal Assays

Date, 1957	Therapy	17-KS,* Mg./24 Hr.	17-OHCS,† Mg./24 Hr.	Creatinine, Mg./24 Hr.
Feb. 19		6.5	9.3	251
20	Repository corticotropin injection, 20 units i. m.	6.1	11.5	117
21		9.3	11.2	162
22		9.1	12.6	118
March 9	Tumor removed			
	Cortisone acetate, 50 mg. i. m., & hydrocortisone, 62.5 mg. i. v.			
10	Cortisone acetate, 50 mg. i. m., & corticotropin, 10 units i. v.			
11	Cortisone acetate, 25 mg. i. m., & corticotropin, 10 units i. v.	1.7	9.0	180
12	Corticotropin, 10 units i. v., & prednisone, 2.5 mg. orally	4.3	9.8	203
13-17	Daily doses of repository corticotropin injection, 5 units i. m., & prednisone, 2.5 mg. orally	3.7	13.1	200
18	No medication			
19	Dismissed	1.3	4.7	129
March 20	Repository corticotropin injection, 5 units twice weekly			
to April 18	for two weeks, then 5 units once weekly for two weeks			

* 17-Ketosteroid assays were performed according to method of **Drekter et. al.** (17)
† 17-Hydroxycorticoid assays were performed according to method of **Reddy et. al.** (18)

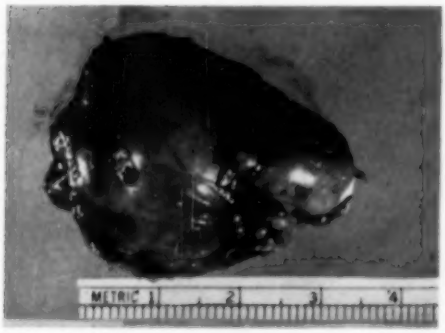

Fig. 2.—Right adrenal tumor removed from infant.

of 5% dextrose in water for injection during the surgical procedure. Also, during the procedure, the patient received 250 cc. of whole blood with 12.5 mg. of hydrocortisone. Following surgery, 50 mg. more of cortisone acetate was administered intramuscularly. The surgical approach was by transperitoneal laparotomy, and a tumor mass replacing the right adrenal gland was removed. The left adrenal was examined and appeared normal. No complication arose during surgery, and the postoperative course was uneventful.

Prophylactic antibiotic therapy was employed during the postoperative period, and no more than a slight transient hyperpyrexia on the second and third postoperative days was noted. The infant received 25 mg. of cortisone acetate intramuscularly twice on the first postoperative day, then 12.5 mg. twice on the second day. Prednisone, 2.5 mg., was given in daily oral doses for the subsequent six days. Ten units of corticotropin (ACTH) was included in the intravenous fluids during the first three postoperative days, then 5 units of repository corticotropin injection was given intramuscularly once daily for the next five days. After the third day the infant began to take fluids adequately and had recovered from a three-day episode of polyuria.

Urinary hormone assays were repeated serially during the postoperative period. Daily values for urinary 17-ketosteroids and 17-hydroxycorticoids fell substantially after surgery, rose transiently during early supportive steroid therapy, then descended toward normal levels 10 days after surgery, when corticotropin and steroid medication had been tapered down in dosages (Table). Clinical improvement following surgery was noted by improved disposition, normal appetite, and regression of the acneform face lesions. The patient was dismissed on March 19, 1957. Repository corticotropin injection, 5 units, was administered twice weekly for two weeks, then once weekly for two more weeks. The child was seen for follow-up study seven weeks later, and decided improvement in appearance and in well-being was evident. The acneform rash had disappeared, and polyphagia, polydipsia, and polyuria were no longer present. The fasting eosinophil count was 94 per cubic millimeter.

Pathological Report.—The surgical specimen was a right adrenal gland weighing 11 gm. and measuring 3.5×3.0×2.5 cm. (Fig. 2). On cut

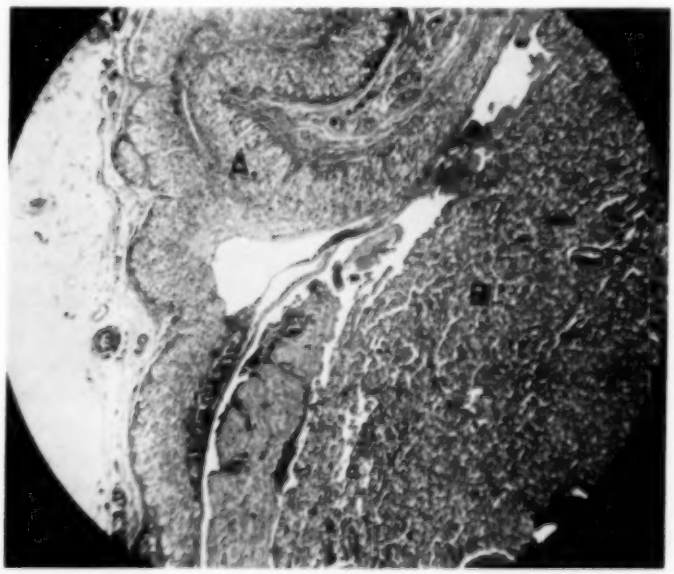

Fig. 3.—Histologic section of adrenal tumor. *A*, normal adrenal cortex; *B*, adenoma.

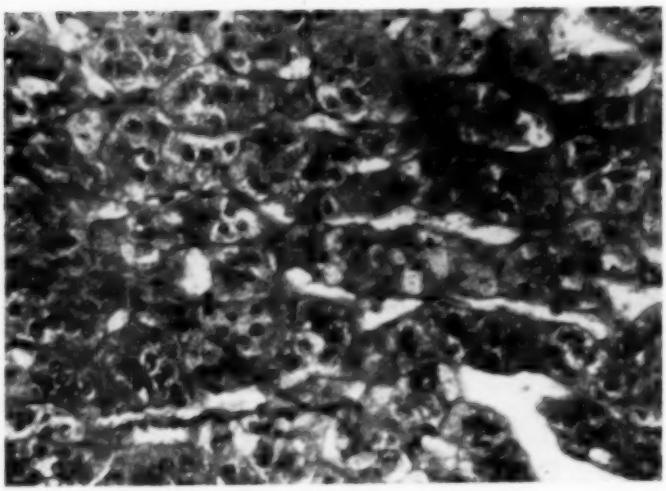

Fig. 4.—Histologic section revealing adenoma. Note arrangement of cells in cord-like fashion simulating the zona fasciculata.

section it appeared that the entire architecture of the adrenal had been destroyed and replaced by a large yellow tumor, which was encapsulated. There were several areas of calcification throughout the tumor. Microscopically, a rim or remnant of compressed normal adrenal cortex was present around the adenoma on one side (Fig. 3). The cells of the cortical adenoma were arranged in cords reminiscent of the zona fasciculata (Fig. 4). There was no evidence of mitosis or invasion. The histopathologic diagnosis was adenoma of the right adrenal cortex.

Comment

Cushing's syndrome in a prepubertal person is rare, and, in infancy, it is almost unique.[1-11] The advanced case presents little diagnostic challenge, but in the very young diagnosis is difficult and the pitfalls are many. Early suspicion of such a condition may be confirmed by hormonal determinations and other diagnostic procedures.[12,13] Newer diagnostic tools developed in the past few years and adequate pre- and postoperative use of corticoids and corticotropins make the problem of hyperadrenalcorticoidism a much easier one to manage now than in former years.

In study of the patient under discussion, the following findings were of importance in establishing the diagnosis: (*a*) The 17-ketosteroids were elevated to 6.5 mg/24 hours. (In our laboratory the normal range for this age group is about 1 mg/24 hours.) (*b*) The 17-hydroxycorticoids were elevated to 9.4 mg/24 hours. (In our laboratory the normal range for this age group is from 1 to 4 mg/24 hours.) (*c*) Circulating eosinophils were persistently absent. (*d*) A faint shell-like calcification in the adrenal area was seen in several flat x-ray films of the abdomen.

The above positive findings, along with the clinical picture of rapid gain in weight, marked mooning of the face, appearance of pubic hair, and slight clitoridal enlargement, warranted the diagnosis of Cushing's syndrome. The polydipsia, polyuria, alkaline urine, and high serum sodium were suggestive of an "aldosterone" component. Corticotropins were administered for two reasons: firstly, to note what the adrenal response would be (it was negligible in this instance, suggesting a tumor rather than hyperplasia [13,14]) and, secondly, to stimulate the contralateral adrenal, which is frequently atrophic when adenoma or carcinoma of one adrenal is present.[15] Descending doses of corticoids and corticotropins were administered postoperatively until it was felt safe to discontinue such medication as judged by the clinical improvement of the patient.[16]

Summary

A case of Cushing's syndrome in a 5-month-old girl is reported. The lesion was a benign adrenal cortical adenoma. Of the 31 instances on record of Cushing's syndrome in children resulting from an adrenal tumor, malignancy was present in 28 and benign adenoma in 3.

Aside from the clinical appearance of marked obesity, moon face, enlarged clitoris, and pubic hair, the diagnosis was facilitated by the elevated 17-ketosteroids (6.5 mg/24 hours) and 17-hydroxycorticoids (9.3 mg./24 hours) and the persistent absence of circulating eosinophils in the fasting blood.

The successful pre- and postoperative management of this infant is described.

Dr. H. F. Hailman, of The Upjohn Company, provided supplies of corticotropin (ACTH), repository corticotropin injection (Depo-ACTH), and hydrocortisone for intravenous administration (Solu-Cortef), and Dr. Edward Henderson, of Schering Corporation, provided supplies of prednisone (Meticorten).

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Congenital Adrenal Hypoplasia

Report of a Case with the Characteristic Clinical Features of Dysadrenocorticism and Autopsy Findings of Extreme Hypoplasia of the Adrenal Glands

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Congenital hypoplasia of the adrenal glands usually is associated with certain malformations of the central nervous system, mainly in anencephalic monsters[1,2] or in combination with other anomalies.[3,4] Much less common is an isolated congenital adrenal hypoplasia. Including his own two cases, Mosier[5] found only five known cases.[6-8] Two of these, however, also had marked hypoplasia of the pituitary gland.

The patient to be described in this case report, a boy who died when he was 33 days old, showed the characteristic clinical features of dysadrenocorticism, and autopsy revealed extreme hypoplasia of the adrenal glands.

Report of Case

The mother was a healthy 22-year-old Rh-positive WR-negative primigravida and primipara. The pregnancy was uneventful; labor was spontaneous, and delivery was uncomplicated. The presentation was cephalic. The full-term baby cried at once, was not cyanotic, and showed no signs of asphyxia. Birth weight was 4090 gm.; length, 54 cm. Immediately after birth, a generalized bronzed pigmentation of the skin, accentuated on the areolae and around the external genitalia, was observed. Since several members of the mother's family were dark-skinned and dark-haired, and inasmuch as the baby showed no active signs of illness, there was no alarm. The boy was breast-fed by his mother and was thriving.

However, at the age of 14 days, it was noticed that the baby was not gaining weight satisfactorily. Thinking the quantity of her milk was insufficient, the mother began to give a supplement of cow's milk formula. When given the first portion of this mixture, the boy vomited and continued to do so after each feeding. Vomiting was forceful but not projectile. The vomitus consisted of gastric contents.

When the vomiting had persisted for several days, the infant became weak and remarkably somnolent at periods.

When her child was 21 days old, the mother consulted a physician. The baby was given atropine methylnitrate (Eumydrin) and frequent feedings consisting of the usual cow's milk formula for seven days, with no effective results. The vomiting continued, and the baby continued to lose weight.

A week later the mother became very alarmed. Immediately after a feeding, her baby had an episode of cyanosis, pallor, sweating, loss of consciousness, and convulsions.

Three days thereafter the infant was admitted to the hospital. The physician's diagnosis was dystrophia and pyloric stenosis.

On admission the boy was 31 days old. Weight was 3650 gm.—440 gm. below birth weight—and the length was 56 cm. There was a generalized bronzed pigmentation of the skin, accentuated on the areolae and around the external genitalia, and a moderate but clear-cut macrogenitosomia. The skin on the back was extraordinarily hairy.

The infant was emaciated and showed signs of dehydration. Otherwise, the usual clinical examination was negative.

After ingestion of food, there were no peristaltic waves and no palpable pyloric tumor, but there was significant weakness and somnolence.

The symptoms and signs were those of adrenal insufficiency, and the clinical course in the ward supported the diagnosis. The infant had frequent Addisonian-like crises, with cyanosis, pallor, sweating, and loss of consciousness. They were noted especially after feeding and also following other "stress situations," such as taking of blood samples, physical examinations, and so forth.

Laboratory Findings.—The blood showed signs of dehydration. Carbon dioxide in plasma was 40 vol. per 100 cc. Urea content of the blood was 124 and 125 mg/100 ml. in two separate samples.

Unfortunately, it was impossible to determine serum concentrations of sodium and potassium before the patient died.

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From Barneklinikken (Children's Clinic) (Chief, Prof. Leif Salomonsen, M.D.) and Institutt for Patologisk Anatomi (Chief, Professor Olav Torgersen, M.D.), Rikshospitalet (Oslo University Hospital).

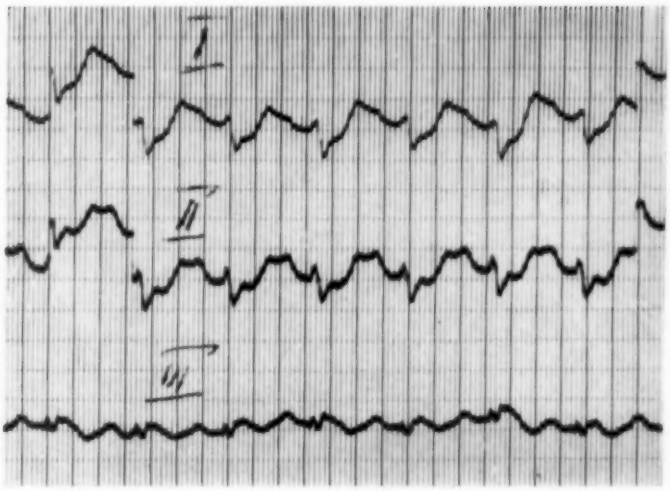

Fig. 1.—An electrocardiogram of the 33-day-old infant with congenital hypoplasia of the adrenal glands.

An electrocardiogram (Fig. 1) taken some hours before death showed decreased voltage of the R waves, depression of the ST segment in standard Leads II and III, and badly marked ST segment in standard Lead I, with almost direct transmission from high S to high T. Such changes are seen in extreme hyperkalemia.

Treatment.—The patient was given parenteral fluid, sodium chloride orally, cortisone, and penicillin. However, being in a very bad condition on admission, the infant died on the second day in the hospital, 33 days old.

Autopsy Findings.—General: An autopsy was performed 21 hours post mortem. The body weight was 3700 gm.; length, 58 cm. The skin was hyperpigmented, most marked on the areolae and the genitalia, which showed macrogenitosomia.

The thymus was rather large, weighing 21 gm. (normal weight in the newborn, 10 to 15 gm.). The weights of all other organs examined (brain, thyroid, heart, lungs, liver, spleen, pancreas, and kidneys), except the adrenals, were within normal limits. This also was true of the hypophysis, which weighed 0.106 gm. (the normal weight in the newborn is about 0.10 gm.).

The adrenals were extremely small (Fig. 2). The right gland was somewhat elongated and weighed 0.176 gm. The left one had a rounded shape and weighed 0.082 gm. Both were soft and were dark red-brown. The size relation between the kidneys and the adrenals is illustrated in Figure 2. Aberrant adrenal tissue could not be found.

Microscopic Examination: Both adrenals revealed the same microscopic appearance. A fibrous capsule surrounded the parenchyme. The whole cortex consisted of large cells, resembling the cells of the fetal zone, without any distinct arrangement (Figs. 3 and 4). The cells were markedly swollen, with ill-defined borders, finely vacuolated and granulated cytoplasm, poorly stained nuclei, and often prominent nucleoli (Fig. 5). No adrenal marrow was found in the usual central position. Serial sections, however, revealed a small eccentric area of normal medullary cells in each adrenal (Fig. 6). None of the glands showed signs of infection or hemorrhage.

The hypophysis showed the usual composition of cells, with normal development of basophils and eosinophils. The other endocrine organs were histologically normal.

Comment

In this case of Addison's disease in a 33-day-old infant, autopsy revealed extremely

Fig. 2.—The small size of the adrenals (total weight, 0.25 gm.) is here seen in relation to the kidneys.

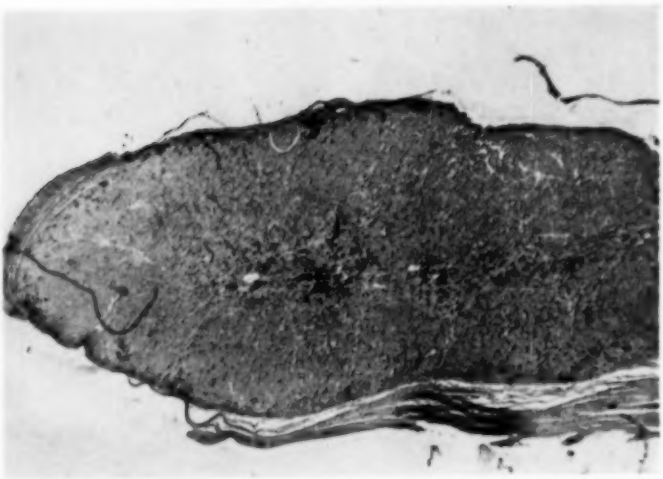

Fig. 3.—Section of the right adrenal gland. No medullary tissue is seen. Hematoxylin and eosin; reduced 8% from mag. × 25.

hypoplastic adrenals, weighing only 0.25 gm., i. e., one-twentieth of the weight of normal adrenals in 1-month-old infants. The cause of his hypoplasia is obscure.

The common pathological basis of Addison's disease is a bilateral destruction of the adrenals caused by tuberculosis or "atrophy." This is true both in adult life and also in childhood. In this case, however, no signs of specific or nonspecific inflammation were found. Neither did the adrenals show evidence of hemorrhage.

Adrenal hypoplasia has been considered to be secondary to the failure of proper development and function of the pituitary gland.[2,9,10] This seems improbable in this patient, because the pituitary gland was of normal weight and structure. The lack of symptoms of deficiency of the the somatotropic and gonadotropic hormones also contradicts this view.

This adrenal hypoplasia seems to have had no relation to adrenal hypoplasias accompanying anencephaly and other similar conditions, since no major malformations of the central nervous system were present. It is worth noting, however, that microscopic ex-

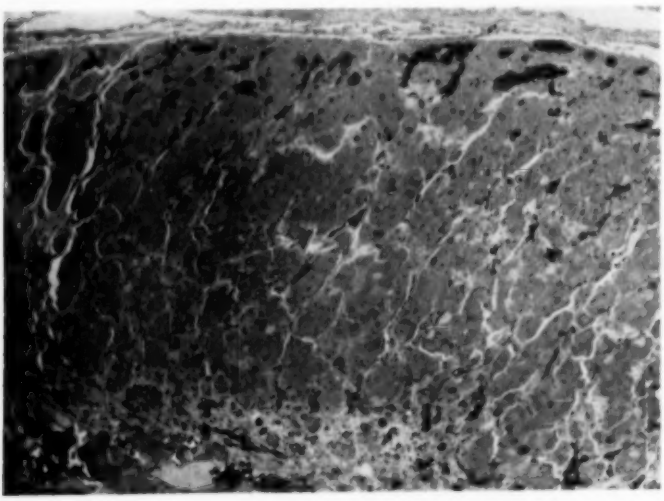

Fig. 4.—The cortex shows no signs of differentiation. Hematoxylin and eosin; reduced 8% from mag. × 100.

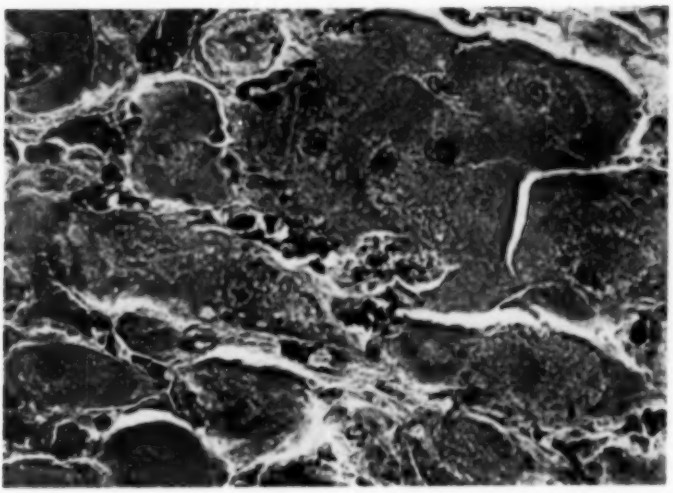

Fig. 5.—Detail from the cortex, showing the swollen cells with degenerative changes. Hematoxylin and eosin; reduced 8% from mag. × 500.

amination of the brain revealed a small dysplastic area in the cerebellum.

On the basis of the histological findings, the possibility of an organogenetic injury of the adrenal glands in early fetal life seems more likely. If one remembers the double origin of the glands with central position of the marrow from the middle of embryonic life,[2,11] the eccentric location of the medulla in both adrenal glands in this case may point to an injury at, or prior to, the time when the cortex is invaded by medullary cells. The mother, however, could tell nothing about any illness at the time when this would have occurred.

The abnormal adrenal appearance with regard to size and structure may explain the deficient growth. The lack of differentiation after birth may also be explained as a failure in response to extrauterine life. At the age of this patient, about one month, the fetal zone is normally involuted [12] and replaced by the permanent zones. Not even the outer zone, glomerulosa, which normally is present already at birth, was developed in this case.

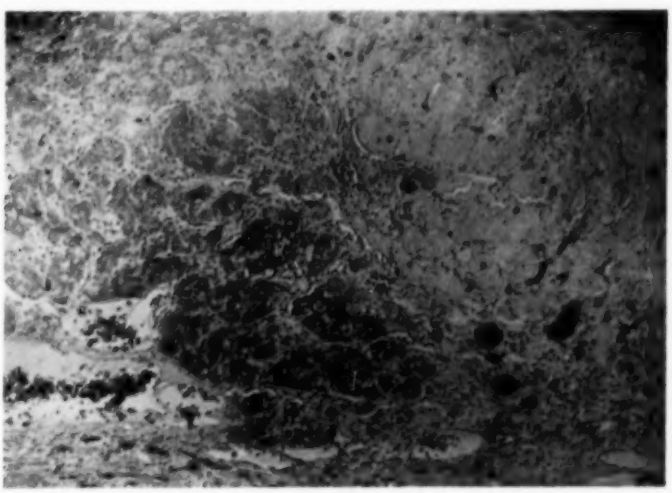

Fig. 6.—The small eccentric area of medullary cells in the left adrenal. Thionine stain; reduced 8% from mag. × 100.

The clinical pattern in this patient may be explained as follows: In fetal life the circulating corticoids probably originate from the maternal adrenals. Migeon and co-workers [13] have studied the placental transmission of adrenal corticoids, measuring the levels in the maternal and cord blood at the time of delivery. They also have administered radioactive hydrocortisone to the mother to measure its transmission. The level in the fetal plasma is always about one-fifth to one-half that of maternal blood, and so there is a partial but not a complete transmission across the placenta. They suggest that at term little or no 17-hydroxycorticosteroids are produced by the fetal adrenals. Their observations are consistent with the findings of Klein and co-workers,[14] that little or no plasma 17-hydroxycorticosteroids are detectable in the first days of life and that normal levels are not achieved until after the first or second week of life.

In accordance with the above-mentioned observations, our patient managed satisfactorily during the first two weeks with—or in spite of—the hypoplastic adrenals. At two weeks the infant for the first time showed signs of illness: vomiting, loss of weight, weakness, and somnolence. It is worth noting that the baby's diet at this time was changed from mother's milk to a cow's milk formula. It may be a coincidence, but we will not ignore the possibility that the change in diet may have acted as a stressor and precipitated the symptoms.[15,16]

The pigmentation of the skin is a well-known sign in Addison's disease and probably is due to increased activity of the pituitary gland.[17] The presence of hyperpigmentation at birth in this case is noteworthy, as well as the familial disposition to hyperpigmentation.

Several hypotheses concerning the macrogenitosomia found already present at birth may be advanced but evidential support can be given to none of them.

Finally, it is probable that the adrenal glands of this infant were damaged rather early in fetal life and that this early lesion made growth and differentiation difficult or impossible. On the basis of the histological findings on the adrenals, only little, if any, function could be expected. This view is supported by the clinical course. The mother supplied the fetus with corticoids in the prenatal period. Soon after birth the infant showed symptoms of adrenal insufficiency, for his adrenal glands produced little or no corticoids. Before long the stress of extrauterine life, possibly accentuated by the change in diet, became too much. Addisonian crises developed, and the infant died in hyperkalemia.

Summary

The characteristic clinical syndrome of Addison's disease in a 33-day-old infant is described. Autopsy revealed extremely hypoplastic adrenals, weighing but 0.25 gm., which is only one-twentieth of the normal weight. The pathogenesis of the hypoplasia and the relation between the hypoplasia and the clinical course are discussed.

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Gonadal Dysgenesis

Bonnevie-Ullrich-Turner Syndrome with Elevated Urinary Gonadotropins in a Nine-Year-Old Child

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Gonadal dysgenesis is being reported with increasing frequency during childhood, but in only four instances [1-3] have elevated levels of gonadotropins been reported in children before the age of 10 years. We wish to report a child with the typical findings of the Bonnevie-Ullrich-Turner form of gonadal dysgenesis in whom elevated urinary gonadotropins were found at the age of 9 years.

Report of Case

A 9-year-old white girl was first seen because of loss of hearing as a result of frequent attacks of otitis media. She had been born at full term, weighing 6 lb. 4 oz. (2840 gm.) and had been 19 in. (48.3 cm.) in length. The mother described the pregnancy as being difficult owing to severe nausea and vomiting. In the third month of pregnancy she had experienced a severe emotional shock when she had found her sister-in-law, who had committed suicide by hanging.

At birth the patient had had webbing of the neck and swelling of the dorsum of the hands and feet, but she was said to have been normal otherwise. During the first week of life she had begun to have loose stools, and this had occurred throughout infancy and the preschool years. It appeared to be caused by "roughage in the diet." The rate of weight gain was slow, and at one year of age she was said to have weighed only 12 lb. (5445 gm.). The swelling of the dorsum of the hands and feet gradually decreased and was scarcely noticeable at the end of the first year, but it became more evident when it was demonstrated by the pressure of shoes. Shortly after her first birthday she began having episodes of otitis media. Purulent discharge from the right ear was present during most of her childhood. When she was 4 years old her tonsils and adenoids were removed. At approximately the same time a diagnosis of celiac disease was made and a diet was prescribed which contained a minimum amount of "roughage and sweets." During the year following this change in diet the stools gradually became normal. At the age of 4½ years roentgenograms of the wrists were reported to the mother as having shown her "bone age" to be two years more than her chronological age. Rubeola, rubella, and varicella had occurred without complications. She had sat alone at approximately 8 months and walked alone at 15 months. The mother reported that she began to talk at the normal time but felt that her speech was always somewhat unclear. Dental development had been precocious; the first permanent molar had erupted at 1½ years of age, and the other three first permanent molars had erupted by the time she was 4 years old. Vision was poor, and she wore glasses. The hearing difficulty fluctuated with the degree of severity of the otitis media, but in spite of this she was doing well in her schoolwork. She ate a normal diet, and this did not produce any diarrhea unless an excessive amount of apple or orange was ingested.

No growth defects, anomalies, or delayed development had occurred in any member of the family. The mother, aged 35 years, was in good health and stated that her menarche had been early. She was pregnant when first seen and subsequently delivered a normal full-term boy. Another sibling, aged 8 years, and the father, aged 40 years, also were reported to be in good health.

Physical Examination.—The patient was a well-developed short, stocky girl who weighed 66½ lb. (30 kg.) and was 47⅛ in. (120 cm.) tall (Fig. 1). Head circumference was 20¾ in. (52.7 cm.), and chest circumference, 27 in. (68.6 cm.). The contour of the head was normal. The hair was thick and fine, with a few gray strands anteriorly. The face was round, with a small mouth that could not be opened widely enough to reveal the anterior surfaces of the teeth (Fig. 2). The mandible was hypoplastic. The ears

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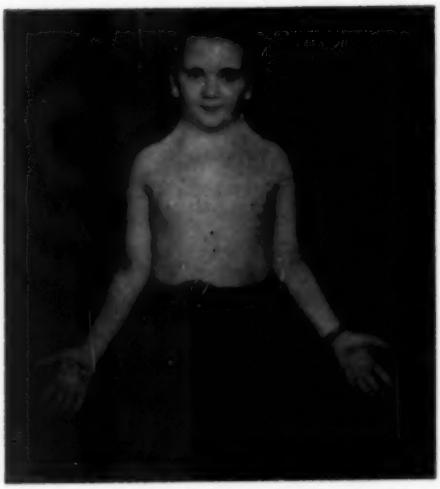
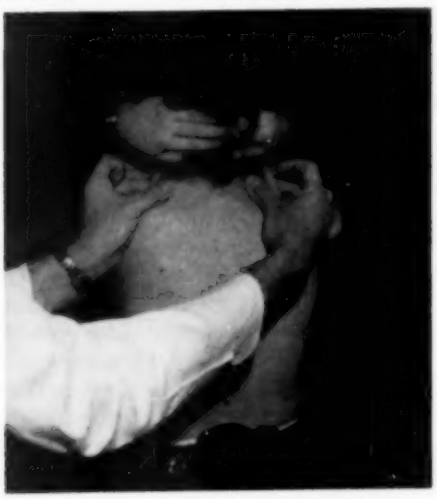


Fig. 1.—Patient with gonadal dysgenesis (Bonnevie-Ullrich-Turner syndrome). Note the feminine appearance, webbed neck, and cubitus valgus.

Fig. 2.—Note the inability to open the mouth widely.

were low-set and small, with large lobes. The left tympanic membrane appeared normal, but the right was dark and irregular. The eyes were normal. The oropharynx was small, and the teeth were carious. Four first permanent molars and two upper and four lower permanent incisors were present. The neck was short, with marked webbing and a low hairline (Fig. 3). The thorax was shield-shaped, with widely separated small inverted nipples. The heart and lungs were normal. The external genitalia appeared to be normal for a prepubescent girl. There was no axillary or pubic hair. Blood pressure was 100/64 mm. Hg in the upper extremities and 130/90 in the lower. The femoral pulses were easily palpated. There was slight cubitus valgus. Both her hands and feet appeared short and broad, with small distal phalanges. Slight swelling of the dorsum of the hands was noted, but no edema of the feet was detected. The toenails were short, with the distal portions raised from the nail bed and the bases deeply set. Numerous moles were distributed over the skin. There was no evidence of cutis laxa, and the skin had normal texture. The remainder of the physical examination was negative.

Fig. 3.—Note the low hairline and webbing of the neck.



Roentgenogram of the chest showed no abnormalities. Epiphyseal maturation ("bone age") was consistent with the chronological age.

A 24-hour urine specimen was assayed for gonadotropins. The urine contained more than 8.8 and less than 35.2 mouse uterine units per 24 hours of urine. Cells obtained by scraping the mucous membranes of the inside of the cheek were examined. Only 2% of them contained sex chromatin.

She was found to have a hearing loss of the conductive type averaging 30 to 35 db. in the right ear and 15 db. in the left ear. Roentgenograms showed right maxillary, ethmoid, and frontal sinusitis and sclerosis of the right mastoid process. She was examined by an ophthalmologist, Dr. Harold Patterson, of Danbury, who found normal visual reflexes and eye grounds but only 20/200 vision in each eye. This could be corrected to 20/25 by a compound myopic astigmatic lens.

Comment

In 1930 Ullrich [4] reported a series of patients who had webbing of the neck or peripheral joints, lymphangiectatic edema of the hands and feet, involvement of the cranial nerves, and muscular aplasias. In some of his patients breast hypoplasia, dwarfism, and skeletal malformations were also present. He suggested that the origin of these congenital anomalies might be similar to those produced experimentally in the mouse embryo by Bonnevie. The syndrome as described by Ullrich subsequently became widely known as "status Bonnevie-Ullrich."

Turner [5] in 1938 reported the cases of seven girls from 15 to 23 years of age with shortness of stature who presented a syndrome of "infantilism, congenital webbed neck and cubitus valgus." Although they were in an age group when sexual maturation might have been expected, their breasts, vaginas, and uteri were infantile and menstruation had not occurred. Although Turner ascribed the changes to a pituitary deficiency, subsequent reports of similar cases in which biopsy of the gonads disclosed characteristic pathologic abnormalities and in whom urinary gonadotropins were increased made it evident that there was a defect involving the gonads. Patients who had such findings were classified as being instances of "Turner's syndrome."

The similarity of status Bonnevie-Ullrich and Turner's syndrome became evident, and in 1950 Ullrich [6] stated that the symmetric form of status Bonnevie-Ullrich described by him was identical with the syndrome of infantilism, webbed neck, and cubitus valgus described by Turner. Various descriptive names have been applied to encompass these conditions, but, until the specific etiologic mechanism has been ascertained, the names "Bonnevie-Ullrich-Turner syndrome," suggested by Rossi [7] to describe the over-all clinical picture, and "gonadal dysgenesis," suggested by Grumbach et al., would appear to be the most appropriate.

The most frequently occurring features of this syndrome in prepubertal children are the following:

1. Congenital webbing of the neck with low hairline at the nape of the neck.

2. Lymphangiectatic edema of the dorsum of the hands and feet. This edema is present at birth and is due to dilatation of lymphatic capillaries. The edema is almost entirely resorbed during the first two years of life, although slight swelling of the hands may be evident for several years.

3. Shortness of stature of moderate degree in many cases.

4. Cubitus valgus (increased carrying angle of the arms).

5. Hypoplastic nipples on a broad shield-like chest.

6. Male sex chromatin pattern in most, but not all, of the apparent females.

7. Various associated anomalies including ocular defects, skeletal and vascular abnormalities, and disturbances of function of the cranial nerves.

8. Various abnormalities of the gonads. These have included "streaked ovaries" composed primarily of fibrous connective tissue, gonads the size of "orange seeds," and, in some cases, gonads that had developed but little, if at all, beyond the stage of the primitive genital ridge.

9. Elevated excretion of urinary gonadotropins. This has been reported mainly after the age of 12 years. Only four cases have had increased amounts of gonadotropins before the age of 10 years.[1-3]

10. Sexual infantilism, with primary amenorrhea, persistence of infantile uterus and vagina, mammary hypoplasia, scanty pubic and axillary hair, and disturbances of hormone excretion including diminished output of urinary estrogen and 17-ketosteroids and increased excretion of urinary gonadotropins, becomes apparent in older persons.

The increased excretion of gonadotropins found in patients with gonadal dysgenesis has been thought to be due to the failure of hormones from the gonads to exert their repressing effect on the pituitary gland, and

on this basis it has been stated that "the lack of ovarian hormones in the female will not become evident until the time of puberty." [8] At the age of 9 years our patient's excretion of urinary gonadotropins was more than 8.8 but less than 35.2 mouse uterine units per 24 hours. In our laboratory, women during the reproductive period of life have been found to excrete between 6.5 and 52 mouse uterine units, and all normal 9-year-old children who have not shown any evidence of sexual maturation excrete less than 2 mouse uterine units per 24 hours. The increased excretion of gonadotropins in our patient and others previously reported indicates that in the younger child titers may be elevated for the age even though concentrations might be within the normal range for women in the reproductive period of life. After the age of 12 to 14 years, when puberty would normally be expected to occur, the concentration of urinary gonadotropins in patients with gonadal dysgenesis will reach levels higher than those found in normal adult women.

In addition to the increased excretion of gonadotropins, our patient demonstrated many of the findings characteristically found in the Bonnevie-Ullrich-Turner form of gonadal dysgenesis. At birth she had had webbing of the neck and swelling of the dorsum of the hands and feet. At the age of 9 years she exhibited shortness of stature, webbing of the neck, and cubitus valgus. Her ears were small and low-set, and the neck was relatively short and had a low hair line. The fifth fingers were short, and there was slight swelling of the dorsum of the hands. The toenails were short and attached only at the base. In addition, she had a small mouth, which could not be opened widely, and numerous moles widely distributed over her body. It was reported that she had had premature eruption of the permanent teeth. Of possible significance was the fact that the mother had vomited excessively during pregnancy and had experienced a severe emotional shock at the end of the first trimester.

The exact cause of gonadal dysgenesis is not known. The most likely basis for the syndrome would seem to be that the embryo is adversely affected by some factor of unknown nature at some time before the eighth to ninth week of pregnancy in the male embryo and somewhat later in the female.[3] Raynaud and Frilley [10] have shown that when removal or destruction of the fetal gonads occurs the subsequent development is invariably along feminine lines. These workers have emphasized the importance of the embryonic testes in counteracting the inherent tendency of the fetus to feminize. Jost [11-13] reported the results of a series of experiments studying the effect of intrauterine castration of rabbit fetuses at successive stages of sex differentiation. When female fetuses were castrated, essentially normal differentiation of the female genital system occurred. However, removal of the fetal testes of the male animals at the same age resulted in feminine development of the genital ridges, urogenital sinus, and external genitalia. When castration was carried out at a later stage, the genital system assumed a progressively more masculine appearance until, finally, castration of male fetuses did not prevent essentially normal male sex differentiation. Following the original work of Jost and Raynaud and Frilley, Wilkins [14] and Jost [12] recognized the clinical significance of these animal experiments and predicted that some patients with so-called ovarian agenesis should be chromosomal males.

More recently, Hoffenberg and Jackson [15] have pointed out that there are certain patients with disturbances in sex differentiation whose abnormalities are not explainable by the above theory alone. On the basis of the work of Witschi,[16] these authors postulate that every person has either a male or a female chemical inductor or "evocator" which exerts a very early influence on the primitive ridge and stimulates its development into either the testis or the ovary. In gonadal dysgenesis this "evocator" may

be absent in some patients. However, this aspect of the subject merits further study.

Studies aimed at determining the sex of persons with gonadal dysgenesis are largely based on the work of Weiman,[17] Painter,[18] and Barr and his associates,[19-21] who described a difference between normal males and females in the nuclear chromatin pattern of various cells. Wilkins, Grumbach, and Van Wyk[22] and Polani, Hunter, and Lennox[23] applied this method of differentiation to patients with so-called ovarian agenesis, and from the evidence obtained by their investigation it was suggested that the basic mechanism in patients with gonadal dysgenesis is the same as that seen in the experiments of Jost where fetal rabbits were castrated at an early stage in development.

The determination of chromosomal sex by a study of cells taken from various tissues of the body offers a valuable diagnostic test in making a definitive diagnosis in the study of apparent females who are stunted in growth or have congenital malformations such as are frequently found in instances of gonadal dysgenesis. Formerly a definitive diagnosis could only be made by peritoneoscopy or laparotomy, although in some cases the finding of an elevated concentration of urinary gonadotropins was suggestive of the diagnosis. Strong presumptive evidence of the diagnosis of gonadal dysgenesis is now possible at any age in suspected persons if it is found that a male sex chromatin pattern is present in those who are clinically female. However, the finding of a female chromosomal pattern does not exclude the diagnosis, since this may occur in approximately 10% to 20% of all patients who exhibit a clinical picture of gonadal dysgenesis. This determination of chromosomal sex is of special value in infants and young children in whom elevated gonadotropin levels may not be present and in whom operative procedures are best avoided. Examination of cells obtained from the buccal mucosa of our patient revealed a sex chromatin pattern that was characteristic of the male. Only 2% of the cells studied showed sex chromatin. In our laboratory normal males have been found to have less than 4% such cells and normal females more than 10%.[24]

Summary

A case of gonadal dysgenesis (Bonnevie-Ullrich-Turner syndrome) with elevated urinary gonadotropins in a 9-year-old child is presented.

The clinical findings of this syndrome are described.

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Abstracts from Current Literature

Presse médicale, Paris

64 (Sept. 1) 1956

* On the Treatment of Infantile Diabetes and Its Prophylaxis. Pierre Mauriac.—p. 1447.
* Therapeutic Activity of Streptomycin Panthothenate in Tuberculous Patients Resistant to Streptomycin. F. Das Neves Almeida and J. M. Das Nevas Almeida.—p. 1452.

65, No. 5 (Jan. 16) 1957

* Artificial Hibernation and Hypothermia in the Treatment of One of Two Siblings with Severe Carbon Monoxide Poisoning. L. Binet, H. Bour, and G. Barrés.—p. 87.

65 (March 13) 1957

* Present Prospects of Immunization Against Poliomyelitis with Attenuated Preparations of Living Virus. P. Lepine and P. Goube De Laforest.—p. 470.

65, No. 23 (March 20) 1957

Infantile Generalized Osteopetrosis (Albers-Schonberg Disease) with Generalized Adenopathy of Hodgkins Type. R. Clement, A. Combes-Hamelle, C. Richir, M. Designolle, J. Poujol, and J. Flieder.—p. 513.

65, No. 24 (March 23) 1957

* Study of Hypoglycemia-Inducing Agents in 275 Diabetic Children. H. Lestradet, J. Besse, and C. Jezequel. —p. 553.

65, No. 26 (March 30) 1957

* Variations of Blood Cholesterol During Episodes of Acute Rheumatic Fever. H. Kaufmann and P. Isorni.—p. 600.

65, No. 30 (April 13) 1957

* A Clinical Study of a New Neuroplegic Agent, 1522 CB (Acetylpromazine Maleate). P. Huguenard.—p. 685.
* Concerning Prolonged Pregnancy. A. J. Bret and J. Barroer.—p. 691.

On the Treatment of Infantile Diabetes and Its Prophylaxis.—In this very interesting review article of childhood diabetes, the author discusses the recent American interest in "free" diet and states that as a matter of practicality few children are kept on a very exact regimen in any country. Thirty years experience has taught him that an intelligent mother, educated by a patient doctor, gradually learns to know better than anyone else how to adapt the dose of insulin to the regimen of her child. The daily treatment of a diabetic child except in exceptional cases has very little to do with science, but much more with observation and good sense. In the author's practice, he has seen only one serious case of acidosis and one case of retinitis. He has, however, seen the syndrome of hepatomegaly, nanism, and obesity (Mauriac syndrome), but he believes that this is an unavoidable complication. He is not of the frequently stated opinion that this syndrome occurs because of therapeutic error, as it may occur in children whose diabetes is well regulated, but he believes that it occurs in children who are in some way predisposed. This syndrome always resolves spontaneously, even in cases where the diabetes remains unregulated. He finds confirmatory evidence for this theory from Dr. Faelli, of the Diabetarium of San Marinella near Rome where 60 child diabetics are cared for from the age of 3 years, under extremely careful control. Among these children, five or six had such unstable diabetes that equilibrium was never attained, and among these hepatomegaly, or a retardation of growth, and in one case a typical Mauriac syndrome have occurred. A diencephalohypophyseal factor is believed to be present in these exceptional cases.

The idea of the prepancreatic period in diabetes is of great importance from the prophylactic point of view. Intermittent glycosuria, transient hyperglycemia, and periods of hypoglycemia may occur which give evidence of a "hormonal machine" which is imperfect. Babies born of diabetic or prediabetic mothers may show high birth weight, adiposity, gigantism, edema, or splenomegaly. These are of such significance that one must always

suspect diabetes in the mothers of such babies. The high mortality of babies born to mothers with severe diabetes is well known, and in 85% Dr. Pricilla White has found hyaline membrane syndrome present at autopsy. Hormonal treatment of diabetic or prediabetic pregnant women with diethylstilbestrol or ethynylestradiol may greatly decrease the infant mortality, but these results of Dr. Pricilla White have not been confirmed by some other workers.

Genetic study in diabetes by Dr. Casa, of the Diabetarium of San Marinella, has shown overt diabetes; simple hyperglycemia, often of a very slight degree; hypoglycemia; albuminuria (50% of diabetic children have one albuminuric relative), and a few cases of optic nerve atrophy in the relatives of 26 children studied. Fifty per cent of the diabetic children studied showed cryptorchism, and 68% of the girls had menarche after the age of 15; all the children showed slow growth but attained normal height after puberty.

In conclusion, the author again states his belief that diabetes is not simply due to pure pancreatic insufficiency but can be better described as a defect in general neuroendocrinosympathetic regulation.

Therapeutic Activity of Streptomycin Panthothenate in Tuberculous Patients Resistant to Streptomycin.—Twenty adult women with advanced tuberculosis progressive in spite of the use of three antituberculous drugs were treated by daily injection of 2.12 to 4.14 gm. of streptomycin panthothenate with isoniazide and PAS. No toxic reactions were noted. Cough, productive sputum, and night sweats were decreased in most of the patients, and the x-ray improved in 50%. The sputum became negative in only 4 of 16 patients, in a follow-up of one month, but this was not surprising in view of the severity of the disease. The effectiveness of this preparation may be due to its activity on strains resistant to streptomycin, its greater diffusion potency, and, lastly, by the availability of a greater amount of streptomycin.

Artificial Hibernation and Hypothermia in the Treatment of One of Two Siblings with Severe Carbon Monoxide Poisoning.—A boy aged 15 and his sister aged 16, victims of severe carbon monoxide poisoning, were studied. The boy died on the fourth day of coma with hyperthermia and convulsions, and his sister, who, with the same picture, appeared on the point of death, was thereupon treated with artificial hibernation (intravenous doses b. i. d. of promethazine [Phenergan], Dolosal, and chlorpromazine) and hypothermia achieved by refrigeration. The girl appeared to improve and lived 10 days longer than her brother in spite of eventually fatal C. N. S. damage. The authors consider this as close to a clinically controlled experiment as could be achieved, and state that the fatal outcome does not condemn the method of treatment but, on the contrary, leads them to ask whether an earlier intervention might not have resulted in cure.

Present Prospects of Immunization Against Poliomyelitis with Attenuated Preparations of Living Virus.—From experiments with this form of immunization, the authors draw the following conclusions: 1. Use of attenuated virus may be considered a promising method. 2. Short-term and irregular immunity may result from use of completely attenuated strains. 3. Strains of high antigenic potency, but without neuropathogenic qualities, should be used, but it is preferable that before using them a basic immunity should be given by immunization with inactivated virus. 4. The interval between the two steps of immunization should probably be longer than 2 months and shorter than 10 months. 5. The duration of the immunity attained is not yet known. 6. The fact that subjects given live virus will be discharging it, even though for a limited period, means that the method must be limited to population areas which have previously received ample basic immunity.

Study of Hypoglycemia-Inducing Agents in 275 Diabetic Children.—Two hundred seventy-five young diabetic boys and girls, aged 3 to 17 years, living in three summer camps for child diabetics, were studied over the months of June, July, and August, 1956, to determine whether the compounds 2254 R. P. and D. 860 would result in any insulin-sparing action. Similar groups received placebo. The degree of insulin-sparing action of these compounds was negligible, and particular cases of diabetes requiring either very small or very large doses of insulin were equally unaffected. This work confirms other studies done with smaller numbers and under less favorable experimental conditions that insulin therapy is still the basic treatment for the child diabetic.

Variations of Blood Cholesterol During Episodes of Acute Rheumatic Fever.—In 12 cases of rheumatic fever, 10 acute and 2 subacute, it was observed that 5 to 10 days after the beginning of clinical improvement the total cholesterol would sometimes double or triple in value over a course of several days, with special increase in the esterified portion, for a period of several weeks. All the patients had been treated with cortisone, which will in itself produce an elevated cholesterol level, but the authors do not believe that this could account for the sudden appearance or for the degree of hypercholesterolemia observed in their patients.

A Clinical Study of a New Neuroplegic Agent, 1522 CB (Acetylpromazine Maleate).—Acetypromazine (1522 CB, or Plegicil) is a new neuroplegic agent which, when given in intravenous doses of 0.5 to 1 mg. per kilogram, produces vasoplegia, postural hypotension, hypothermia, tachycardia (sudden, marked, of slight duration, and without apparent ill-effects), absence of respiratory depression, hypokaliemia, hyperexcitability of striated muscle, somnolence, and thirst. More questionable effects are hypokaliuria in 60%, hypocalcemia in less than 50%, relative constancy of Na, decrease in O_2 consumption observed in 50%, and slight changes in terminal portion of the ECG. The drug appears to be twice as active as chlorpromazine and has about one-half of its toxicity in the doses (0.5 mg. per kilogram) needed for equivalent physiologic effects.

Concerning Prolonged Pregnancy.—In this review article of prolonged pregnancy the authors comment on the similar statistics of four recent studies from The Johns Hopkins Hospital and from hospitals in Oslo, Stockholm, and Uppsala. The normal duration of pregnancy is believed to be on the average about 273 days after conception, or 280 days, plus or minus 10 days, from the first day of the previous menstrual period. A prolonged pregnancy is considered to be one lasting more than 294 days from the first day of the last menstrual period (provided the date of ovulation is not difficult to fix owing to long irregular menstrual cycles of the mothers) and occurs in 11% to 12.4% of all pregnancies (data from 13,109 pregnancies), 13.5% of primiparae, 10.8% of multiparae, and 17.4% of women over 40 years. The fetal mortality in primiparae (3694 cases) was 7.2% from 294 to 300 days and 18.5% in pregnancies of 300 or more days. The problem of interruption of a prolonged pregnancy is therefore important, and an accurate method of diagnosis other than anamnestic is greatly needed. Ezes (*Ann. endocrinol.* 14:462, 1953) studied 16 pregnancies lasting 15 to 35 days after normal term and noted two groups. In six cases hormonal studies were normal, the vaginal pH was less than 5, the Doderlein bacilli were abundant and normal, and the caryopycnotic index of the vaginal cells was less than 12%. The uterus continued to grow, the fetal movements were normal, and normal hypermature babies were born. In 10 cases certain differences were noted in the course of the ninth month. The urinary gonadotropins were between 750 and 2000 I. U.; the phenolsteroids, between 5000γ and 8000γ; the pregnanediol, not above 35 mg.; the vaginal pH was above 5; the Doderlein bacilli, short and granular; the vaginal cells, diminished in number, ragged, and showing rounded-up "postnatal cells" of the type described by Papanicolaou; the caryopycnotic index was between 15% and 10%. Clinically the uterus ceased to grow, the fetus moved less, the fetal heart rate varied, and the baby sometimes died in utero or during labor. If he survived birth, he was long, but not heavy, and in one-half of the cases showed serious neurologic difficulties. Since the results of interrupting pregnancies on the basis of prolongation diagnosed by anamnesis have generally been inconclusive, further such studies leading to an accurate method of diagnosis are needed.

Radiology, Syracuse, N. Y.

68 (April) 1957

Congenital Goiter. W. William Nagle, John W. Hope, and Alfred M. Bongiovanni.—p. 526.

Meconium Ileus. Ronald E. Herson.—p. 568.

Bilateral Osteochondritis of the Middle Cuneiform Bone. Ivor P. Smyth.—p. 575.

Congenital Goiter.—This is an account of three female infants with swelling in the neck from birth. Dyspnea, cyanosis, and choking spells were prevalent symptoms. One mother during her last pregnancy had a basal metabolism rate exceeding +50%. The mother of a second girl had received 5 grains (325 mg.) of potassium iodide three times daily because of a basal metabolism rate of +32%. The mother of the third was not known to have received any goitrogenic foods or drugs or suffered from thyroid disease.

A thyroidectomy was performed in one case, and 80% of the thyroid gland was removed. It was necessary to maintain the child on desicatted thyroid, but at 18 months the child was normal in all other respects.

In congenital goiter there is usually a soft symmetrical mass. The infant's head and neck are frequently extended to obtain a better airway. There may be a low hoarse cry. Roentgenograms made in full inspiration show the soft tissue mass surrounding the trachea, sometimes the esophagus, compressing and displacing these structures. The cervical spine may show reversed curvature.

The majority of infants born with thyroid enlargement do not show evidence of thyroid hormone abnormality. Only two cases of hyperthyroidism are on record.

Surgical intervention may be necessary to prevent death from asphyxia. Tracheotomy alone may not be satisfactory, since this may fail to relieve the retrotracheal obstruction.

Maternal thyroid disease and ingestion by the mother of goitrogenic drugs and the goitrogenic action of calcium in low-iodine areas, as well as the geographic "goiter belts," were considered important etiological factors. The exact mechanism responsible for the development of congenital goiter remains unknown. An increase in the incidence of neonatal goiter may be anticipated because of the widespread use of goitrogenic drugs during pregnancy.

Meconium Ileus.—An analysis is made of 17 cases of meconium ileus encountered at at Babies Hospital, New York, from 1945 through 1955 and proved by surgery or necropsy. All roentgenograms except two were made in the upright lateral and supine positions. Evidence of distal small bowel obstruction was present in all but one case on examinations made at four and eight hours after birth. The colon was usually demonstrable at 12 hours, but the roentgen diagnosis was hazardous before 24 hours unless failure of progression of air could be demonstrated on serial studies.

Intestinal obstruction caused by inspissated thick ropy tenacious meconium adhering to the mucosa was usually a manifestation of cystic fibrosis of the pancreas. In other types of neonatal intestinal obstruction, such as atresia, bands, volvulus, and duplication of the intestinal tract, the intestinal contents orad to the site of occlusion were made up of normal meconium. Peritonitis occured where the intestinal tube was defective or perforated and meconium escaped into the peritoneal cavity (complicated meconium ileus).

The usual signs are progressive distention of the abdomen, vomiting, and failure to pass meconium. All of these signs may not be present. Palpation of the meconium determines with high probability the nature of the ileus.

Volvulus of the midintestine is frequently associated with meconium ileus and may progress to strangulation, gangrene, perforation, and peritonitis.

The roentgen signs that have been reported were investigated. One, the bubbly appearance (Neuhauser) caused by swallowed air mixed with meconium was present in 12 of the author's 17 cases and, while nonspecific, was considered of value. Another sign, consisting of the absence of fluid levels in dilated loops of small bowel, indicating the presence of uncomplicated meconium ileus (White), was not dependable in the author's group.

News and Comment

ANNOUNCEMENTS

Pediatric Orthopedics.—A five-day program in pediatric orthopedics will be jointly presented by the departments of pediatrics and orthopedics of Tulane University School of Medicine between the dates of Feb. 24 and 28, 1958. Dr. William T. Green, of Harvard Medical School, will assist the combined faculties in dealing with diagnostic and nonoperative features in the management of congenital disturbances, metabolic disorders (including orthopedic reflections of systemic disease), malignant and other bone tumors, cerebral palsy and allied conditions, and functional, postural, and developmental problems. Lectures as well as small group discussions of patients and round-table seminars will be used in presenting material. As usual, registration is restricted to forty qualified specialists.

Further information and applications may be secured from the Division of Graduate Medicine, Tulane Medical School, 1430 Tulane Ave., New Orleans 12.

Books

BOOK REVIEWS

The Mentally Retarded Patient. By Harold Michal-Smith. Price, $4.00. Pp. 203. J. B. Lippincott Company, 227-231 S. Sixth St., Philadelphia 5, 1956.

This book attempts to coordinate the areas of vital conflict in the adjustment of the mentally retarded patient. It offers to the physician a well-rounded picture of the mentally retarded person as viewed psychologically, from the family situation, and as viewed from the problem of educablity and vocational training. The main emphasis lies on the psychological aspects of retardation, and, as such, it gives a fairly complete picture of both the disabilities and the potentialities. The author shows in considerable detail how the retarded person may fit into a social pattern to the benefit of himself in society, if he is permitted to develop within the limits of his maximal ability. In these aspects, the book is both general and specific and may well serve as a guide for the private physician in need of help in the over-all care of the mentally retarded.

The classification of mental retardation given in this book is that of the Clinic for Mentally Retarded Children of the Flower and Fifth Avenue Hospitals. According to this classification, those patients without known neurological malformation or specific nervous system disease would be classified as familial defect or as an undifferentiated cerebrocranial defect. The classification is of extreme interest to the physician in that it permits him to realize the broad scope of diseases which are associated with retardation and should encourage him to think in terms of etiological classification when confronted with a problem of mental retardation. It follows from proper classification that advice to the parents regarding future pregnancies might become extremely important, and awareness of the underlying pathology should serve in many instances to afford opportunities of prevention mental retardation. Metabolic abnormalities such as galactosemia and phenylpyruvica oligophrenia are pointed out as instances where dietary treatment may be of value. More emphasis might be placed on the role of the private physician in preventing mental retardation secondary to acquired disturbances of the central nervous system, such as damage from erythroblastosis, dehydration, and acute bacterial infection of the central nervous system.

The greater portion of this book deals primarily with children who would be classified as retarded on the basis of a familial defect or undifferentiated cerebrocranial defect. These children without neuromuscular disabilities and specific neurological defects who have an impaired ability to learn can certainly be well trained and become well adjusted in a modified protected environment, as Dr. Michal-Smith points out. However, the bulk of patients with mental retardation appeared to this reviewer to be in the categories associated with neuromuscular and neurological disabilities. The impairments of hearing, speech, and locomotion, in addition to the deficit of abstract thinking, generally prove the most persistent barriers to successful adjustment of the patient. A more comprehensive picture of the mentally retarded in regard to his handling might result from additional discussion of the neurological and orthopedic background of the disabilities along with some guide to long-range training to overcome the specific handicaps.

Pediatric Cardiology. By Alexander S. Nadas. Price, $12.00. Pp. 587, with 343 illustrations. W. B. Saunders Company, 218 West Washington Sq., Philadelphia 5, 1957.

This is a book devoted primarily to a discussion of the clinical aspects of heart disease in children, both congenital and acquired. As is pointed out by the author, it does not purport to go into physiologic action of the heart, except as such information is valuable in the diagnosis and management of cardiac disease; certain tools of diagnosis such as cardiac catheterization and electrocardiography are, however, very adequately explained on physiologic principles.

The book is divided into four parts. The first part is concerned with the techniques of diagnosis, in which are included chapters on (1) history, physical examination, and routine tests; (2) radiology of the heart; (3) electrocardiography; (4) phonocardiography; (5) cardiac catheterization, and (6) angiocardiography. This material, including the section on history and physical examination, will prove valuable not only to the inexperienced student of

pediatrics and heart disease but to the more mature clinician as well. Part 2 includes a discussion of acquired heart disease with chapters on acute rheumatic fever and rheumatic heart disease, bacterial endocarditis, arrhythmias, myocardial disease, diseases of the pericardium, congestive failure, and miscellaneous diseases of the heart. In Part 3 is found the discussion of congenital heart disease; this presentation abandons the conventional classification, based on cyanosis, and presents an anatomical classification in which are found three main divisions, as follows: (1) communication between systemic and pulmonary circuits with left-to-right shunt; (2) valvular and vascular lesions with a right-to-left shunt or no shunt at all, and (3) the transpositions. The material in this section is very well presented, with the clinical picture clearly described along with the characteristic findings in more specialized investigations. Catheterization data are particularly clearly presented in a diagramatic form. Part 4 is a discussion of anesthesia for children with heart disease prepared by Robert M. Smith, M.D., Chief Anesthesiologist, The Children's Medical Center, Boston.

The material presented in this volume largely represents the author's own experience and views, with much of the clinical material derived from series of cases seen at the Boston Children's Hospital. It is inevitable that exceptions will be taken in several instances by cardiologists with a different experience. In really controversial issues, however, a discussion of the several points of view with appropriate bibliography is given. This volume would thus appear to be a highly valuable addition to pediatric literature.

The Metabolic Response to Neonatal Surgery. By Peter Paul Rickham. Price, $5.00. Pp. 93, with 22 illustrations. Harvard University Press, Cambridge 38, Mass., 1957.

In an effort to understand more completely the metabolic consequences of major surgical procedures in very young infants, extensive metabolic studies were done in nine infants of various ages in the immediate postoperative period. The results of these investigations are presented in detail in this volume. Also included is a review of other work concerning the metabolic consequences of surgery in the adult and older child and a summary of existing knowledge of normal metabolic processes in the newborn infant.

After correlation of all the above material plus experience gained with many other infants undergoing surgery, but without such extensive metabolic studies, recommendations are made as to optimal methods of management of the very small infant in the postoperative period. These include considerations of maintenance of body temperature, caloric intake, fluid and electrolyte requirements, nitrogen requirements, and postoperative laboratory control.

The number of infants studied is admittedly small, but this investigation seems very carefully done and the derived recommendations appear sound. This volume should be of great interest to all physicians involved in the care of very young infants undergoing surgery. It should also prove a stimulus for further, more extensive investigations along the same lines.

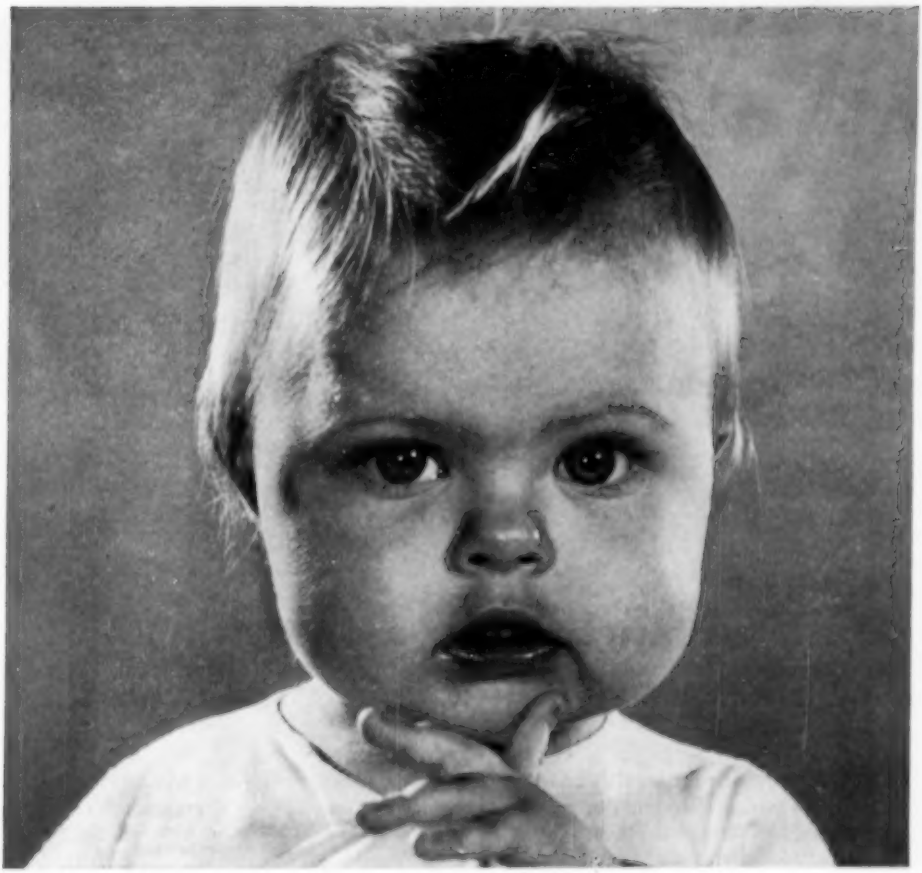
Histamine: Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Price, $9.00. Pp. 472, with one illustration. Little, Brown and Company, 34 Beacon St., Boston 6, 1956.

This book presents the papers given in April, 1955, during two symposia on histamine in honor of Sir Henry Dale. The British Pharmacological Society and the Physiological Society, supported by the Wellcome and Ciba Foundations, arranged the meetings. An international group of those whose main interest is research on histamine was invited. The papers are divided among three general subjects: (1) the occurrence of histamine in the body, (2) the release of histamine, and (3) the origin, significance, and fate of histamine in the body.

The first symposium consists of 15 essays and 20 short communications. The second consists not only of the nine essays given formally but also a transcript of the discussion which followed each presentation and a final over-all discussion.

The material cannot be summarized briefly, but attention may be drawn to some of it. The widespread distribution of histamine in the body closely parallels the mast-cell population of the various tissues. Many substances release histamine in vivo and in vitro. The factors which inhibit release of histamine in anaphylaxis are becoming better understood. Stimulation of gastric secretion is a definite function of histamine. Our knowledge of enzymology as it relates to histamine metabolism is increasing.

This volume is an excellent source book on histamine metabolism and physiology not only because of its content but also because it has been so capably edited.



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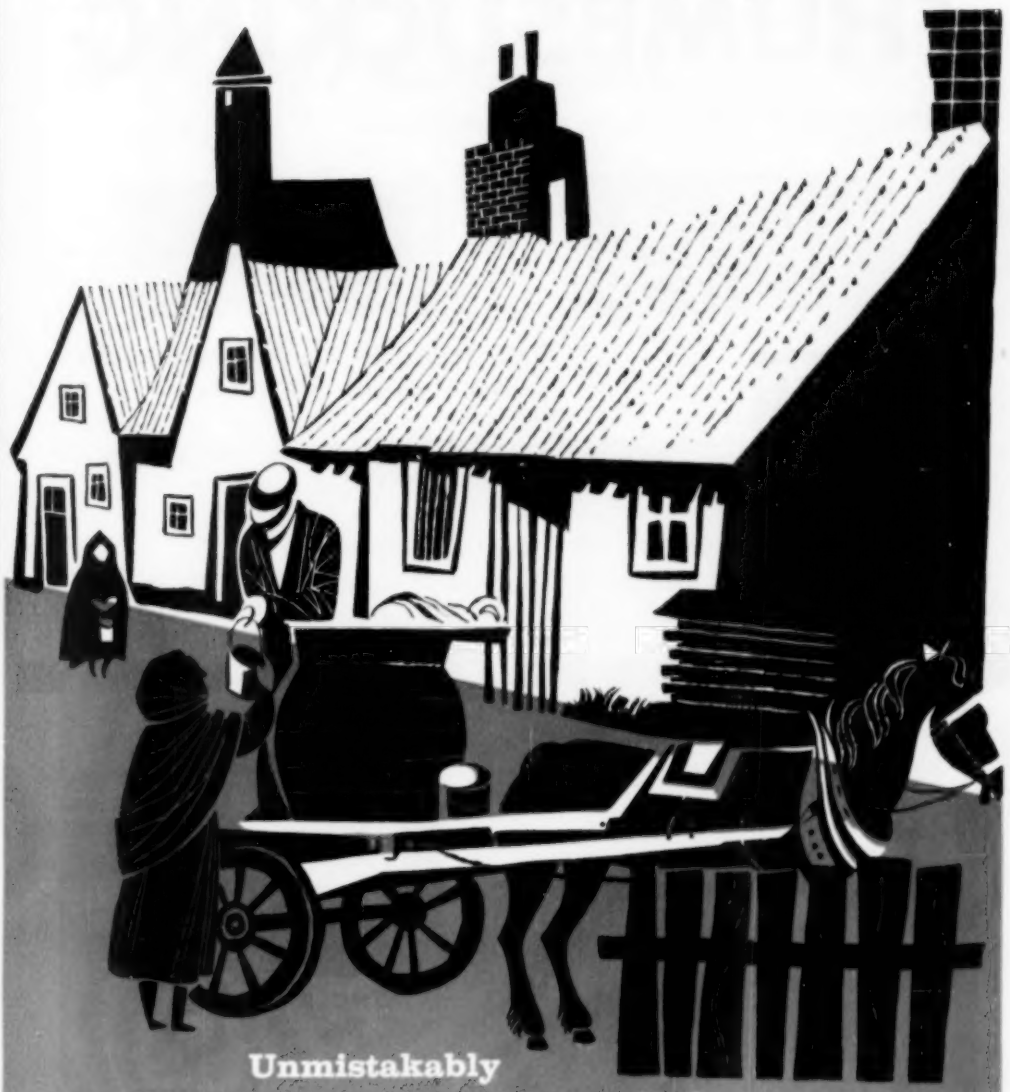

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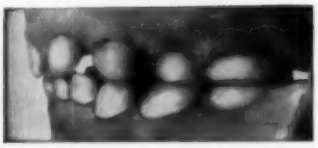

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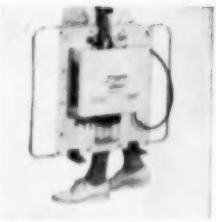

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